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PROGRAM FOR ACTA MEDICA SCANDINAVICA 1973

In the year 1973 new editors will take over the journal after Birger Strandell, who has been in office for 7 years. It would now seem appropriate that we should discuss possible changes and —we hope—some improvements for the years to come.

Many modern medical journals have editorials on some topic of special current interest. We know however from the experiences and complaints of many medical editors, that it is difficult to get people who are willing to write such editorials. On the other hand it cannot be denied that a good editorial may be quite important both as information for the reader and in order to mark a certain policy in scientific matters. Our hope is that each number of the journal may contain a short editorial, if possible connected with the topic of some papers in that number The editors will do their best to find persons who are willing to make such contributions, but we are of course glad if we can receive such papers without asking.

The present policy of the journal has seemed to be rather restricted in accepting case reports. We think, however that a concentrated report on an interesting case with brief remarks and a few well chosen quotations from the recent literature may be of great value. We are therefore happy if we can receive such short papers and our aim would be to have one case report in each number Another special type of casuistics might be to publish short case histories under the title "What happened to the patient? We have a feeling that many authors publish their data on a patient long before the final outcome is known. It might well be that the further development will show that the first diagnosis was wrong or that the later development of the disease was quite important for an understanding of the whole picture. One of the difficulties with this type of publication is bibliographic. We shall try to overcome such problems if it is found that the readers of the journal will contribute to this section.

The system of referees came into being during the last part of 1972. The referees will remain strictly anonymous and we hope that they will be willing to return manuscripts to the editorial office without delay The opinion of the referee should be written on a special form accompanying the manuscript One of the great advantages of the referee system is that an author may improve his manuscript according to the hints given. Each author will therefore get the referee's opinion if the manuscript is sent back for re-editing. We suspect, however that there will be many manuscripts which do not need any changes and will be accepted in their original form. This new system means that the function of the "Redactores" from the different countries will be somewhat changed. These eminent colleagues are expected not only to stimulate the delivery of manuscripts suitable for publication in Acta medica scandinavica from the various countries, but, it is hoped, to function as referees even in the future.

There are, of course, many other dreams that a new editor may have, but we think it wiser not to promise too much from the beginning. One interesting development would be to have a guest editor taking care of one number He should be specially interested in the subject treated in the majority of the papers, but we do not think that this number should contain exclusively papers on a single subject. In such numbers the guest editor would be invited to write a more comprehensive editorial as an introduction.

We hope that we shall be able to carry on the traditions of our journal and enable it to continue as the voice of Scandinavian internal medicine among the ever increasing number of medical periodicals.

Malmö, October 21 1972

Jan G Waldenström

Editor

Harry Boström

Ass. Editor

Lars Erik Böttiger

Ass. Editor

Acta med. scand. Vol. 193 pp. 3–4 1973

Editorial

LESSONS FROM THE STUDY OF MYELOMA AND ALLIED CONDITIONS

One of the most difficult problems in the treatment of tumour patients is the quantitation of effects. The measurement of tumour size is a difficult question for many reasons, one of the main ones being the unfavourable relation between diameter and volume.

The first use of a specific metabolite produced by tumour cells for the evaluation of therapeutic effects was when Huggins followed the level of acid phosphatase in the blood during treatment of patients with metastasizing prostatic carcinoma. This has later become the prototype for such checking of tumour regression.

During the last years a number of other metabolites from malignant tumours have been studied for this purpose. The excretion in the urine of hydroxyacetic acid, HIAA, as a breakdown product of 5-hydroxytryptamine (serotonin) 5-HT in carcinoid tumours is an excellent example. Both acid phosphatase and 5-HT are produced by the normal cells that give rise to the tumour Their origin is therefore topical, i.e. they are formed in the normal tissue from which the tumour originates. In recent years ectopic formation of chiefly polypeptide hormones has been the subject of much study These substances may be regarded as metabolites, formed by derepression of dormant templates that are awakened in carcinoma cells from many different organs such as kidney lung, ovary etc. If a primary tumour without metastases is removed, this ectopic production is stopped.

Myeloma is a unique tumour insofar as it produces topical protein products, immunoglobulins and parts of such molecules, light chains or Bence Jones protein. These proteins are secreted into the body fluids. The formation of these substances is a good indication of the vitality of the tumour Their blood levels may therefore be used in order to follow progression or regression after therapy The fact that each myeloma patient produces only a single immunoglobulin molecule is important for more theoretical reasons. This homogeneity of the product can be determined in many ways. The study of markers on the molecule, e.g. the Gm groups or other immunologically detectable characters, has been the chief method. It seems probable that all cells in myeloma tissue are derived from one cell, later forming a clone. This concept of monoclonality has had a great importance also for clinical diagnosis. Myeloma globulins and macroglobulins have therefore been studied in great detail by a large number of workers, and it may well be said that most of our knowledge regarding the chemistry of these substances stems from studies on such patients. In recent years a number of mouse myeloma strains have also become the object of biochemical study All these investigations are the basis for the recently acquired knowledge regarding the general structure of an immunoglobulin molecule as developed by Porter and others. Porter was able to prove that the molecule consists of heavy and light chains. Edelmann was able to prove that the light chain was identical with the Bence Jones protein found in many myeloma patients. Later these chains were studied by many biochemists, who used the product from different myeloma patients. Thus the complete sequence of amino acids in many Bence Jones proteins was established by many authors and interesting conclusions could be drawn from their findings. The importance of these findings has been recognized by the awarding of the 1972 Nobel prize for physiology and medicine to Edelmann and Porter Finally Edelmann succeeded in the determination of amino acid sequences in a complete myeloma globulin. This was the first antibody to be analysed in complete detail.

The question now arises: Are these products of malignant, i.e. of tumour cells real immunoglobulins, are they functioning antibodies? The

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BONE MARROW BIOPSY IN DIAGNOSIS OF WHIPPLE'S DISEASE

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Abstract. It is known from autopsy studies that the bone marrow often contains cells typical of Whipple disease. This knowledge has, however, apparently never been utilized for diagnosis of the condition intra vitam. Typical cells have now been found in bone marrow smear. Electron microscopy of post mortem tissue from the bone marrow confirmed the presence of bacterium-like structures in histiocytes.

Whipple's disease offers diagnostic problems. The clinical manifestations vary from case to case, and many organs may be involved. Since it is now possible to control the disease with antibiotics, it is of paramount importance to diagnose the condition intra vitam. This is usually possible by examination of intestinal biopsy specimens, which should be done by light and electron microscopy because a definite diagnosis requires demonstration of bacterium-like inclusions in histiocytes. The presence of PAS-positive histiocytes in the intestinal wall does not in itself warrant a diagnosis (4). There are cases in which a correct diagnosis has been made by lymph node biopsy (4). Rectal biopsy is usually not diagnostic. In 1962 Aust and Smith (1) reported a case with bone marrow involvement at autopsy. The authors proposed that bone marrow biopsy might be used for diagnosis in suspect cases of Whipple's disease. It has been shown in large autopsy series that the bone marrow is often involved (6).

A search of the literature failed to reveal any case in which bone marrow smears, obtained intra vitam, had permitted a diagnosis. Such a case, however is described below. The bone marrow findings were originally misinterpreted, and so were the autopsy findings owing to some atypical staining features. But on review several years later the correct diagnosis was established and electron microscopic examination of an autopsy specimen of the bone marrow revealed bacterium-like inclusions in the bone marrow histiocytes. This is the first occasion on which these inclusions have been electron microscopically demonstrated in bone marrow histiocytes. Readers interested in a comprehensive discussion of Whipple's disease are referred to recent reviews (e.g. 3, 4).

CASE REPORT

The patient, 73-year-old woman, had rheumatic fever at the age of 20, but no sequelae. She underwent uterine amputation at the age of 47. One ovary was left. Since the age of 49 she had suffered from keratoconjunctivitis sicca. At 62 she was admitted to hospital because of Ménière's disease. The hematological data were normal. At 72 she was also examined in hospital because of parkinsonism. Blood values were still normal.

Apart from gallstone, for which cholecystectomy was performed in 1957 the patient had never had any abdominal trouble until Dec. 1964, when she suddenly fell ill, with abdominal tenderness and fever (38°C). In two months she lost 6 kg. In Jan. 1965 she was admitted to hospital. Physical examination was unrewarding: no hepatosplenomegaly or lymphadenopathy. X-ray examinations of the stomach, colon and urinary tract revealed nothing remarkable. Paper electrophoresis showed albumin 1.1 g/100 ml, α_1 0.39 g/100 ml, α_2 0.54 g/100 ml, β_1 0.37 g/100 ml, β_2 0.25 g/100 ml and γ 0.35 g/100 ml. The electrophoretic pattern of the urine was normal. Comprehensive serological examination demonstrated extremely low antibody titres, which were interpreted as signs of immunological incompetence. No antinuclear factors. GOT and GPT normal, while the alkaline phosphatases were slightly raised. Tests for occult fecal blood were always negative except once when it was slightly positive. Culture of urine gave growth of Klebsiella and Proteus. Culture of feces revealed no growth of Salmonella, Shigella or staphylococci. One blood culture showed no growth at all.

Hematological values. Hb 13–15 g/100 ml. RBC 4.5–5 mill./mm³. WBC 1 000–2 000/mm³. The differential count always showed less than 5% lymphocytes. The monocytes varied between 5 and 15%. No eosinophils.

An occasional myelocyte and plasma cell was seen. There was also thrombocytopenia with platelet counts between 75 000 and 90 000 mm³. Smears of sternal bone marrow were made. An occasional large reticulum cell of obscure significance was observed (vide infra). Lymphography revealed enlarged retroperitoneal lymph nodes with pathological structure. A tentative clinical diagnosis of reticulum cell sarcoma was made.

Further course. The patient developed pneumonia, which as successfully treated with   tetracycline preparation. It was, however, only given for one week. She was also treated with steroids, initially prednisone, 40 mg a day. Fever promptly disappeared. She was sent home on prednisone 20 mg   day but no other therapy.

She was reexamined one month later. She still had no diarrhea, but frequent vomiting. Prednisone therapy was continued and the blood values successively became better. The leucocyte count was normalized, but the lymphocyte count never xceeded 15%. The platelet count bordered the lower limit of the normal range. The patient nevertheless continued to lose weight and became excessively tired. Candida infection of the throat also appeare., and the prednisone dose was reduced to 10 mg a day.

At the beginning of July she was in a very bad condition and was readmitted to hospital. The fever had recurred. A blood culture showed growth of Proteus. Tetracycline were given and the steroid dose was doubled but the patient died within   few days.

Autopsy. Emboli in the pulmonary artery. Microscopically there was widespread pneumocystis pneumonia. The spleen weighed 600 g, while the liver was not enlarged. The intestines ere of normal ppearance and no tissue as saved for microscopical examination. Many mesenteric lymph nodes were enlarged and had a yellow surface. The nodes along the aorta were markedly   d. The naked-eye appearance of the bone mar was normal. Microscopically the red pulp of the   as massiv ly infiltrated with large histiocytes, whic were rather eakly PAS-positive. The liver contained some similar cells and the bone marrow large foci. Many peripheral and abdominal lymph nodes were examined microscopically and all contained large numbers of histiocytes. The mesenteric nodes were partly destroyed by these cells and embedded in histiocytic granulomas and fibrotic tissue. Whipple disease was considered but excluded because of the weak staining with PAS in the pleen. No definite diagnosis could be made.

Several years later the case was brought to the attention of the author. When reviewing the bone marrow slides, histiocytes were seen to contain bacterium-like inclusions typical of Whipple's disease. They were not easy to discern in hematoxylin-eosin stained slides and the tissue blocks from the autopsy were sectioned and stained afresh. Smears of sternal bone marrow obtained intra vitam were also reviewed. Luckily un stained smears were available.

METHODS

Histological sections of paraffin block were stained with hematoxylin and eosin, PAS, Giemsa, Gram and silver methenamine according to standard procedures.

Smears were stained with PAS according to the routine of the laboratory. Smears were stained with a modified silver methenamine technique in the following way. After fixation for 30 min in 96% ethanol the smear was stained with methenamine silver according to Gomori but with a prolonged time (3 hours) in the silver solution.

Tissue for electron microscopy was prepared in the following way. From the paraffin block of femoral bone marrow obtained at autopsy a 1 mm³ piece was cut from   part known from the light microscopical sections to contain many "Whipple cells". It was deparaffinized in xylene and rehydrated through a decreasing series of ethanol, postfixed in 1% OsO in   phosphate buffer dehydrated in ethanol and embedded in Vestopal. Ultrathin sections were mounted on grids and examined in an electron microscope.

RESULTS

The histological sections stained with PAS showed the histiocytes to be strongly positive in the bone marrow (Fig. 2 *b*) and the lymph nodes, but only weakly positive in the spleen. Sections stained with silver methenamine showed large amounts of black rods in the histiocytes in the bone marrow the lymph nodes and the spleen. The rods were consistently Gram-negative. Giemsa staining made the cells appear blue-black because of the large number of rods (Fig. 2 *a*). The bone marrow smears originally stained with May-Grünwald-Giemsa contained large histiocytes with many rod-like bodies (Fig. 1) with a violet hue. About ten such cells were found in each smear (not very cellular). The cells proved to be strongly PAS-positive also in the smears. Silver staining beautifully demonstrated the rods intra cellularly (Fig. 3) and surprisingly large amounts of fragments of cytoplasm presumably from histiocytes, with the bacterium-like rods all over the smear and also numerous extracellular rods of identical morphology. The cytoplasmic fragments and extracellular rods were not easy to recognize in the May-Grünwald-Giemsa stained smears, but were easy to find after silver staining, as the rods proved to be negatively stained. Similarly treated bone marrow smears of comparable age from patients with other hematological diseases did not show such rods.

The electron microscopic sections contained many histiocytes with rods of bacterial morpho-

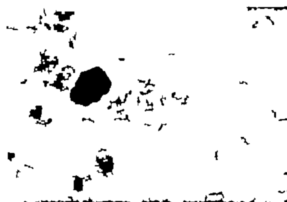


Fig. 1 Histiocyte with rod-like inclusions in bone marrow smear. May Grünwald-Giemsa, 800.

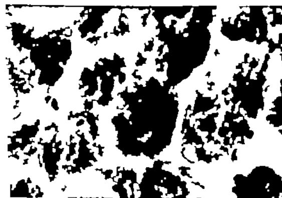


Fig. 2. (a) Section of necropsy specimen of femoral bone marrow Numerous histiocytes ith rod-like inclusions. Giemsa, 800.

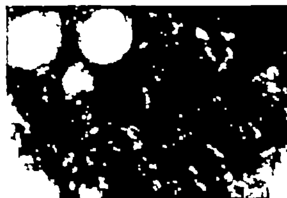


Fig 2 (b) Section of necropsy specimen of femo marrow PAS, 320.

fut-mit mod. %

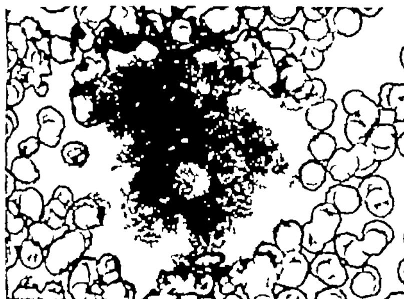


Fig. 3. Histiocyte with rod-like bodies in bone marrow smear. Modified silver methenamine, × 900.

logy (Fig. 4). The rods were mostly situated in vacuoles in the cytoplasm, but the cell organelles were badly preserved and it was not possible to draw any conclusions concerning the relation of the bacteria to other intracytoplasmatic structures. Some were seen to penetrate the cytoplasma membrane, others made deep invaginations in the nuclei.

DISCUSSION

Whipple's disease seems to require some sort of symbiosis between histiocytes and bacteria. The exact nature of the bacteria is not known. Material obtained by bone marrow puncture should be ideal for cultivation if the organisms are present. The disease does not seem to be infectious in the ordinary sense of the term. Some factor in the host may be necessary to establish the disease. The cells are probably disseminated from the intestinal lymph nodes to other organs harbouring the bacteria. The outspoken focal character of the cell collections in the bone marrow might indicate that the cells proliferate in situ, possibly transmitting the bacteria from one generation to the next. Electron microscopic studies of biopsy material from the intestines have shown that the bacteria are to a certain extent degraded in the histiocytes. Nonetheless the condition is fatal if left untreated. The ba[illegible] a biological advantage o[illegible] immunological deficiency m[illegible]cessary for the establishmen[illegible] fective blast transformation [illegible] has been described (5). Leucope[illegible] lymphocytopenia, is sometime[illegible] et al. (4) could trace only three [illegible] gammaglobulinemia in the lite[illegible] [illegible] possib[illegible] that lymphocytes and gammaglobu[illegible] re lost [in] the intestines and the destruction [of] the nodes may be partly responsible for the lymph[ocyt]openia and hypogammaglobulinemia.

In the case described above the lymphocytopenia and hypogammaglobulinemia probably develop secondarily to the disease. The pneumocystis carini pneumonia shows that at the end the patient's resistance to infection was low. The literature contains reports of other cases of Whipple's disease in which the patients had infections with organisms of low virulence, such as progressive multifocal leucoencephalopathy (2).

This report stresses the importance of bone marrow examination in cases suspect of Whipple's disease. Cells like those described above in malabsorption states argue in favour of Whipple's disease. Bone marrow biopsy is simple and not time-consuming, while intestinal biopsy is possible only in specialized centres. Further it is



Fig. 4 Electron micrograph of bone marrow demonstrating histiocyte ith bacteria. Uranyl acetate and lead citrate 9 000.

stressed that the reaction of autopsy specimens to PAS is unreliable. The report also shows that ectron microscopy may be used in the post diagnosis of Whipple's disease.

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Acta Med. scand. Vol. 193 pp. 9–21 1973

OCCURRENCE OF DIABETIC TYPE OF PLASMA FFA AND GLYCEROL RESPONSES TO PHYSICAL EXERCISE IN PREDIABETIC SUBJECTS

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Abstract. The mobilization of glycerol and free fatty acids (FFA) from fat stores during physical exercise has been studied in 6 patients with insulin-dependent diabetes, 3 with diabetes in clinical remission, 10 with prediabetes and in 11 healthy control subjects. They were all submitted to the same relative exercise test calculated as about 60% of the maximal capacity All subjects with manifest diabetes, 4 of the prediabetics and one of the diabetics in remission, but none of the controls, demonstrated exaggerated increases in plasma glycerol and FFA during the exercise test. There were no consistent differences between the groups as regards the changes in glucose, insulin and growth hormone (GH) in plasma during exercise. The findings of elevated plasma glycerol and FFA could not be attributed to subnormal increase in the lactate levels during exercise. A possible explanation of the increased lipolytic response might be that in certain prediabetics the diminished insulin response to hyperglycemia results in decreased antilipolytic capacity of adipose tissue. The increased lipid mobilization in some of the prediabetics seems to add another feature to this phase of diabetes.

Diabetic subjects demonstrate several abnormalities in lipid metabolism, one of the manifestations of which is excessive mobilization of lipid from adipose tissue. This alteration is probably due to the deficient insulin secretion and is corrected by insulin administration.

We have previously demonstrated that a deficiency in insulin response to glucose may also occur in some subjects with normal glucose tolerance (13 14). We consider this condition of deficient insulin response concurrent with normal glucose tolerance as prediabetes. The aim of the present study was to determine whether this insulin deficiency in prediabetics could be compatible with normal lipid mobilization. Physical exercise was used as a tool in these studies, since it is known to stimulate markedly the mobilization of lipids from fat stores (1 5 19 25). Since plasma growth hormone (GH) concentration is altered by exercise (21 27 28 29 40) the level of this hormone was followed. The response of the above parameters to exercise being partly dependent on the working load (6, 7 38 39 47), it seemed pertinent to use in all individuals the same relative work load, calculated as a given percentage of the maximal working capacity In addition to prediabetic subjects the studies were performed on normal subjects, patients with juvenile diabetes mellitus and diabetics in spontaneous clinical remission.

MATERIAL AND METHODS

The four groups of subjects studied were: 1) 11 subjects with unimpaired insulin response to glucose and normal glucose tolerance, 2) 10 subjects with subnormal insulin response to glucose and normal glucose tolerance (prediabetics), 3) 6 patients with diabetes mellitus of the juvenile type, and 4) 3 patients with diabetes mellitus in clinical remission. (Table I.)

The two groups of healthy subjects, i.e. the prediabetics and normals, were all volunteers, selected from larger group with normal glucose tolerance and divided into two groups according to the plasma insulin response to glucose infusion (13 14) In Table I are recorded the k values of the i.v. glucose tolerance test and the k_{IT}-values (see below) reflecting the amount of insulin released initially as function of hyperglycemic stimulation.

Mild to moderate ketonuria but no metabolic acidosis was recorded in five diabetic patients newly discovered and treated with diet for few days in the hospital. They were later discharged on insulin therapy The sixth diabetic had been satisfactorily controlled with diet alone after the initial period of insulin treatment However, three weeks after the present study this patient was admitted to the hospital with severe hyperglycemia and moderate ketonuria. No other endocrine disorders or acute illnesses observed in these six diabetic patients.

Table I. *Clinical data, insulin response to glucose infusion (k_B), i.v. glucose tolerance (k-value), and circulatory evaluation in the subjects studied*

Subject no.	Sex	Age (y)	% of ideal b.wt.	Glucosuria on day before the study (g/24 h)	Ketonuria	Insulin dose at discharge (IU NPH)	k_B	k-value (%/min)	W_{170}	Work load at experiment (kpm/min)	Total Hb (g)	Blood volume (l)	Heart volume (ml)
Controls													
1	♂	[illegible]	96				2.04	1.22	1 150	650	644	6.3	705
	♂	33	97				2.38	1.98	1 050	700	680	5.6	772
[illegible]	♂	24	89				2.54	1.31	1 150	650	899	6.5	595
4		17	93				2.37	3.64	550	425	—	—	—
5		22	109				2.51	[illegible]	600	375	553	5.2	670
6		25	100				[illegible]	1.90	750	550	412	4.2	570
		[illegible]	9				2.23	1.40	900	600	498	4.0	500
8		33	89				[illegible]	3.15	550	350	478	4.1	465
9	♂	24	104				[illegible]	2.4	900	650	716	5.0	750
10	♂	25	94				—	2.39	1 800	1 100	813	6.4	880
11	♂	37	113				2.44	1.93	900	600	648	4.6	795
Prediabetics													
1	♂	[illegible]	115				1.76	1.22	1 000	650	794	6.6	895
13	♂	24	93				1.86	1.58	750	550	578	4.7	805
14		40	105				1.70	1.54	650	425	484	5.9	600
15	♀	44	97				1.77	1.22	550	400	364	3.3	345
16	♂	27	99				1.20	1.67	1 200	800	832	6.8	805
17	♂	33	108				1.91	1.32	1 300	900	790	6.3	955
18	♂	19	95				1.46	1.40	900	600	536	4.0	—
19		34	104				1.89	1.54	650	450	470	3.6	625
20	♂	[illegible]	94				1.75	1.14	900	650	744	5.4	705
1		34	83				1.87	1.58	650	450	430	3.9	500
Diabetics													
22	♂	32	89	305	–	28			550	400	616	5.6	630
	♂	32	105	150		32			800	500	641	5.3	760
	♂	18	92	40		28			700	425	727	4.7	840
	♂	17	90	not recorded		[illegible]			1 050	750	649	5.2	765
[illegible]6		[illegible]	[illegible]	167	+ + +	20			450	350	444	3.8	470
[illegible]	♂	[illegible]	[illegible]	150	+ +	28			600	400	590	4.6	565
Diabetics in remission													
28		19	104					0.74	800	500	481	5.5	610
[illegible]		33	108					—	650	400	455	4.4	710
30		16	96					0.43	750	400	462	4.2	—

The three patients with diabetes mellitus in clinical remission had been treated with insulin, and one of them had earlier also been given oral antidiabetic therapy (pat. 30). The treatment was withdrawn in these three subjects [illegible], 9 and [illegible] months, respectively prior to the investigation. One had slightly elevated fasting blood glucose concentration on the day of the study. The glucose infusion test was not performed in these subjects.

All routine laboratory tests in the patients gave normal results. Furthermore, they all had normal body weight (34). The groups of prediabetic and normal subjects were fairly well matched with regard to age, sex, and body weight. The working capacity with the subjects in the sitting position was determined on an electrically braked bicycle ergometer (Elema-Schönander) (43, 44, 50). The maximal working capacity (W_{170}) was calculated as the rate of work at a heart rate of 170 beats/min. In all subjects the working capacity was normal. Although the W_{170} tended to be lower in the diabetic patients (692 ± 87 mean ± S.E.) than in the control subjects (936 ± 109), there was no statistical difference. Also the heart and the blood volume (43, 44, 50) were found to be normal within two weeks before the present study. The work load used in this study was selected to represent about 60–70% of the W_{170} during the whole period of exercise.

All subjects were in the fasting state from about 9 p.m. on the day before the study. None of the hospitalized patients were allowed to leave their bed in the morning before the investigation. The whole study including the period of exercise, was performed with the subjects in the supine position. A teflon catheter was inserted percutaneously into a brachial artery and kept patent with repeated small injections of saline. After a 1-hour rest the subjects exercised for 20 min at a rate of 60 r.p.m.

Table II. *Pulse rate and concentration of lactate (mean ± S.E.) in arterial blood before and during exercise in the four groups of subjects*

Statistical significance is calculated for the exercise-induced changes with each group (p_1) as well as between the control group and the three other groups (p_2). NS = statistically not significant

	Before exercise		Min after start of exercise 11			18		
	Lactate	Pulse rate	Lactate	ΔLactate	Pulse rate	Lactate	ΔLactate	Pulse rate
Controls ($n = 11$)								
Mean	0.59	76	4.74	+4.14	140	4.85	+4.26	145
S.E.	0.07	3	0.53	0.52	4	0.56	0.53	4
p_1				<0.001	<0.001		<0.001	<0.001
Prediabetics ($n = 10$)								
Mean	0.59	69	4.23	+3.64 ($n = 9$)	135	4.47	+3.88	139
S.E.	0.07	4	0.67	0.68	3	0.82	0.84	3
p_1				<0.001	<0.001		<0.005	<0.001
p_2				NS	NS		N.S.	NS
Diabetics ($n = 6$)								
Mean	0.61	77	2.73	+2.12	129	2.48	+1.86	135
S.E.	0.08	7	0.43	0.45	9	0.30	0.33	9
p_1				<0.01	<0.001		<0.005	<0.001
p_2				<0.02	NS		<0.001	NS
Diabetics in remission ($n = 3$)								
Mean	0.55	66	2.69	+2.14	130	3.02	+2.47	135
S.E.	0.10	3	1.14	1.11	12	1.20	1.15	2
p_1				NS	<0.001		NS	<0.001
p_2				NS	NS		NS	NS

The exercise period was followed by 1-hour rest. ECGs recorded before, during and after the exercise showed no abnormalities.

At intervals indicated in the tables and figures 17 samples of arterial blood were taken in heparinized syringes. Arterial blood for lactate analysis was drawn 5 min before and 11 and 18 min after the start of exercise, and lactate was determined in duplicate by the enzymatic method of Hohorst (26). Four aliquots of blood were rapidly pipetted off for duplicate determinations of glucose by commercial oxidase method (Kabi, Sweden), and the remainder was immediately chilled in ice until the end of the experiment.

After centrifugation aliquots of the plasma were processed for determination of free fatty acids (FFA) and glycerol. The Dole procedure (17), as modified by Trout et al. (49), was used for determination of FFA, and glycerol was measured according to the coupled enzymatic procedure of Wieland (52) as modified by Larsen. Plasma stored at −20°C was later used for analysis of insulin and GH according to the double antibody techniques described by Hales and Randle (20) and Cerasi et al. (12). The i.v. glucose tolerance test was performed according to Ikkos and Luft (30).

The insulin response to glucose infusion was calculated by analogue computation (11). The k_G-values obtained and used for discriminating prediabetics from the healthy controls (Table I) reflect the magnitude of the early insulin response in relation to the degree of glucose stimulation.

Statistical analysis was carried out according to Snedecor (45).

RESULTS

The mean pulse rate and the levels of serum lactate before and during exercise (at 11 and 18 min) are presented in Table II. No significant differences were observed between the control subjects, the prediabetics and the diabetics in clinical remission. It is noteworthy that the increase in serum lactate during exercise was less pronounced in the diabetic group than in the control subjects ($p < 0.02$ and < 0.001), although the changes in the pulse rate were of the same order of magnitude.

From Table III and Fig. 1 it can be seen that *the plasma glycerol response* to exercise differed markedly between the control subjects and the diabetic patients. In the latter group there was a rapid rise in glycerol far above that of the other

Table V. *Concentrations of blood glucose (mg/100 ml) and plasma insulin (μU/ml) (mean ± S.E.) before, during and after exercise in the four groups of subjects*

	Preexercise				Exercise					Postexercise							
	Min before start of exercise				Min after start of exercise					Min after start of exercise							
	−30	−15	−10	−5	5	8	11	15	18	21	25	30	35	40	50	60	80
Controls (n = 11)																	
Blood glucose																	
Mean	67.7	67.3	67.8	68.5	69.8	68.0	63.5	62.5	62.3	62.7	65.5	68.9	65.8	68.9	69.9	70.4	71.5
S.E.	3.4	3.1	3.4	3.3	3.7	4.1	4.3	4.6	4.0	4.2	3.9	4.5	4.3	3.0	3.7	3.7	3.3
Plasma insulin																	
Mean	17.8	17.2	16.3	16.2	18.6	16.5	16.0	16.0	15.5	15.0	16.9	15.9	15.0	15.7	17.2	16.2	17.4
S.E.	2.4	2.2	1.9	1.2	2.7	2.6	2.4	2.2	1.8	2.6	1.8	1.4	1.8	1.7	2.2	2.3	2.0
Diabetics (n = 6)																	
Blood glucose																	
Mean	193.4	192.2	189.0	187.2	187.8	189.0	180.4	175.2	167.2	163.8	168.0	172.8	169.4	168.2	170.0	172.4	164.2
S.E.	7.9	7.3	8.7	7.8	6.5	10.0	7.2	6.6	6.0	7.6	6.4	9.2	7.9	8.1	8.2	7.9	6.7
Plasma insulin																	
Mean	12.0	12.8	13.0	11.8	11.5	12.8	12.0	11.7	11.3	12.2	12.3	13.8	12.2	12.0	13.0	11.6	12.2
S.E.	1.3	2.0	2.2	1.8	1.3	1.8	1.6	1.5	1.9	1.2	1.8	1.6	1.6	1.8	2.2	2.5	2.1
Prediabetic (n = 10)																	
Blood glucose																	
Mean	70.8	69.3	68.9	68.7	68.9	70.0	64.7	63.1	62.7	62.6	63.0	63.0	66.0	65.2	64.6	64.7	65.8
S.E.	2.4	2.0	2.1	2.4	2.3	3.2	3.4	3.2	3.4	3.9	3.5	4.3	4.3	4.2	5.0	4.0	4.3
Plasma insulin																	
Mean	14.8	13.7	14.0	14.0	13.4	14.6	13.2	14.0	14.4	14.2	15.0	12.9	13.9	14.4	14.2	15.3	13.8
S.E.	1.8	1.5	2.2	1.4	0.8	0.8	1.1	0.9	1.0	1.6	1.7	1.9	2.0	1.9	1.0	1.9	2.0
Diabetics in remission (n = 3)																	
Blood glucose																	
Mean	92.7	85.7	83.7	82.3	82.6	88.3	83.6	79.3	78.0	75.0	74.3	81.7	80.7	78.3	78.3	78.3	79.0
Range	74–1.5	70–110	68–109	69–106	70–107	77 110	72 104	68–99	65–99	64–93	62–93	67–99	65–100	62 100	60–100	61 102	62 104
Plasma insulin																	
Mean	20.5	21.0	20.2	20.0	18.7	20.3	19.2	19.7	20.0	19.7	18.2	18.0	18.7	19.0	19.3	19.0	21.0
Range	19–22	18–23	18–23	19–22	18–20	19–21	18–20	18–22	18–23	18–22	17 20	16–19	17 20	17 20	18–21	18–20	19–24

trols and prediabetics. These differences were not significant. No clear change in plasma insulin was observed during exercise in any of the groups.

As seen in Table VI, the subjects in the control group had normal GH values at rest and a moderate GH peak at 25–30 min after the start of the exercise. Higher resting values and a marked GH response to exercise were observed in two prediabetics (pats. 16 and 21), who also had exaggerated FFA and glycerol responses. On the other hand, normal resting and exercise values were obtained in the other prediabetics, including two of those (pats. 14 and 19) who showed a marked increase in glycerol and FFA concentration during exercise.

The preexercise levels of GH were increased in all the diabetics. In three subjects (nos. 22, 24 and 25) a slight and gradual decrease in GH occurred during exercise, whereas the remainder exhibited a peak at different times after the onset of the work load. Subject 26 alone showed an impressive increase in plasma GH.

In the group of diabetics in remission high initial GH values were recorded in subject 30 who also had a slightly elevated blood glucose concentration. Subject 28 who showed a marked glycerol and FFA response, and subject 29 did not differ in GH values from the controls.

DISCUSSION

It has been clearly demonstrated in this study that, in insulin-dependent diabetic subjects, physical exercise is accompanied by an immediate and marked rise in the arterial concentrations of FFA and glycerol. These findings are in agreement with those reported previously by Carlström *et al.* (8 9 10) The new finding in the present study is the demonstration of a diabetic type of lipid mobilization in 4 out of 10 subjects defined as prediabetics. As already mentioned, these individuals are characterized by the same type of deficient insulin discharge on glucose administration as diabetic subjects but, in spite of this, they retain a normal glucose tolerance. Obviously these 4 prediabetics behaved as diabetics at the level of adipose tissue, but as normals from the point of view of overall glucose metabolism.

We have previously shown that the conversion of pyruvate to glucose is normal in prediabetics, whereas oxidation of pyruvate to CO_2 is decreased as in diabetes (42). Thus it seems as though the prediabetics behaved as normal subjects at the site of the liver while being diabetic" in the periphery Since glucose tolerance is mainly regulated by hepatic mechanisms (18) the prediabetic subjects, in toto, remain non-diabetic. The increased lipid mobilization described here adds another peripheral diabetic feature to the prediabetic individuals.

The abnormal lipid pattern during exercise is most likely due to accelerated mobilization of lipids from fat depots. This is supported by several indirect findings in the literature: that the turnover of FFA and glycerol is increased in the depancreatized dog (22, 31)· that the clearance of glycerol is not significantly decreased in diabetes mellitus (37) and that there seems to exist a close relationship between the concentration of glycerol in plasma and the turnovers of FFA and glycerol in conditions with augmented mobilization of fat from adipose tissue (7 23 39).

It is of interest that signs of increased lipolysis (22, 23 24) during exercise did not occur in any of the 11 normal subjects but only in those with deficient insulin secretion, all of the diabetics, 4 out of 10 prediabetics and one out of 3 diabetics in clinical remission. Therefore it would seem likely that insulin deficiency is one of the main causes of augmented lipolysis in these patients. On the other hand the basal levels of plasma insulin were not decreased in the prediabetics and diabetics in remission. Furthermore, plasma insulin was not increased in the normals during exercise. Therefore, if insulin lack is causative factor for increased lipolysis, it will have to be attributed to some long-term effect of consistently subnormal *insulin response to carbohydrate intake, which* would result in reduced antilipolysis at the adipose tissue level. It has to be remembered that insulin production is more deficient in juvenile diabetics than in prediabetics and mature-onset diabetics, and probably also than in diabetics in remission (2, 14 36, 41). This may partly explain why all diabetics, but only a minority of the other two groups, showed increased lipolysis.

Increased lipolysis during exercise might also be due to increased adrenergic stimulation. However no significant differences were noted in the cardiac responses (pulse rates) of the different groups of subjects to exercise. It was recently reported that the concentration of norepinephrine in blood during exercise increased to higher levels in

Table V *Concentrations of blood glucose (mg/100 ml) and plasma insulin (µU/ml) (mean ± S.E.) before, during and after exercise in the four groups of subjects*

	Preexercise				Exercise					Postexercise							
	Min before start of exercise				Min after start of exercise					Min after start of exercise							
	−30	−15	−10	−5	5	8	11	15	18	21	25	30	35	40	50	60	80
Controls (n = 11)																	
Blood glucose																	
Mean	67.7	67.5	67.8	68.5	69.8	68.0	63.5	62.5	62.3	62.7	65.5	68.9	65.8	68.9	69.9	70.4	71.5
S.E.	1.4	1.1	1.4	1.3	3.7	4.1	4.3	4.6	4.0	4.2	1.9	4.5	4.3	3.0	3.7	3.7	3.3
Plasma insulin																	
Mean	17.8	17.2	16.3	16.2	18.6	16.5	16.0	16.0	15.5	15.0	16.9	15.9	15.0	15.7	17.2	16.2	17.4
S.E.	2.4	2.2	1.9	1.2	2.7	2.6	2.4	2.2	1.8	2.6	1.8	1.4	1.8	1.7	2.2	2.3	2.0
Diabetics (n = 6)																	
Blood glucose																	
Mean	193.4	192.2	189.0	187.2	187.8	189.0	180.4	175.2	167.2	163.8	168.0	172.8	169.4	168.2	170.0	172.4	164.2
S.E.	7.9	7.3	8.7	7.8	6.5	10.0	7.2	6.6	6.0	7.6	6.4	9.2	7.9	8.1	8.2	7.9	6.7
Plasma insulin																	
Mean	12.0	12.8	13.0	11.8	11.5	12.8	12.0	11.7	11.3	12.2	12.3	13.8	12.2	12.0	13.0	11.6	12.2
S.E.	1.3	2.0	2.2	1.8	1.3	1.8	1.6	1.5	1.9	1.2	1.8	1.6	1.6	1.8	2.2	2.5	2.1
Prediabetics (n = 10)																	
Blood glucose																	
Mean	70.8	69.3	68.9	68.7	68.9	70.0	64.7	63.1	62.7	62.6	61.0	63.0	66.0	65.2	64.6	64.7	65.8
S.E.	2.4	2.0	2.1	2.4	2.3	3.2	3.4	3.2	3.4	3.9	3.5	4.3	4.3	4.2	5.0	4.0	4.3
Plasma insulin																	
Mean	14.8	13.7	14.0	14.0	13.4	14.8	13.2	14.0	14.4	14.2	15.0	12.9	13.9	14.4	14.2	15.3	13.8
S.E.	1.8	1.5	2.2	1.4	0.8	0.8	1.1	0.9	1.0	1.6	1.7	1.9	2.0	1.9	1.0	1.9	2.0
Diabetics in remission (n = 3)																	
Blood glucose																	
Mean	92.7	85.7	83.7	82.3	82.6	88.3	83.6	79.3	78.0	75.0	74.3	81.7	80.7	78.3	78.3	78.3	79.0
Range	74–123	70–110	68–109	69–106	70–107	77–110	72–104	68–99	65–99	64–93	62–93	67–99	63–100	62–100	60–100	61–102	62–104
Plasma insulin																	
Mean	20.5	21.0	20.2	20.0	18.7	20.3	19.2	19.7	20.0	19.7	18.2	18.0	18.7	19.8	19.3	19.0	21.0
Range	19–22	18–23	18–23	19–22	18–20	19–21	18–20	18–22	18–23	18–22	17–20	16–19	17–20	17–20	18–21	18–20	19–24

Diabetics in remission (n=3)																	
28	7.4	5.8	5.8	6.2	8.0	11.5	8.4	10.0	8.6	9.0	7.6	6.4	7.8	5.8	6.4	5.2	8.4
29	5.4	5.5	4.3	4.6	5.6	5.2	5.7	4.9	5.5	6.0	6.5	6.5	7.8	6.2	8.1	5.4	5.1
30	*26.0*	*14.5*	*11.5*	*11.0*	7.8	7.6	6.8	9.6	11.5	10.5	12.5	9.4	9.8	9.6	9.8	7.0	4.8
Mean	12.9	8.6	7.2	7.3	7.1	8.1	7.0	8.2	8.5	8.5	8.9	7.4	8.5	7.2	8.1	5.9	6.1
S.E.	6.6	3.0	2.2	1.9	0.8	1.8	0.8	1.6	1.7	1.3	1.8	1.0	0.7	1.2	1.0	0.6	1.2
Δmean					−0.1	+0.8	−0.3	+0.9	+1.3	+1.2	+1.6	+0.2	+1.2	−0.1	+0.8	−1.4	−1.2
S.E.					1.5	2.5	2.0	1.5	0.6	1.0	0.2	1.0	1.3	0.9	1.4	1.4	2.6
P_1					NS	NS	NS	NS	NS	< 0.10	< 0.001	NS	NS	NS	NS	NS	NS
P_2					NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

diabetics than in normal subjects (33) This finding is of limited value for the present discussion, since in the cited paper no account was taken of the individual working loads. In the present study in which the working load was always related to the maximal working capacity of the individual, a major variation between individuals regarding sympathetic activation is less probable.

Although the exercise-induced increase in the blood concentration of lactate was significantly lower in the diabetics than in the controls, there was no evidence of inverse correlation between the changes in lactate versus glycerol when the data of all subjects were analysed statistically It is therefore unlikely that the inhibitory action of lactate on lipid mobilization is of any significance for the interpretation of our findings. The present observation of a less pronounced increase in the concentration of blood lactate during exercise in the diabetic patients than in the control group is in consonance with the results of Wahren et al. (51), showing that the fractional removal of lactate by the splanchnic tissues occurs more rapidly in the diabetics.

Factors such as obesity (3) and physical fitness (32) which may influence the concentrations of FFA and glycerol may be ignored due to the design of the study and the selection of the subjects. The working load used was, by definition related to the physical fitness of the subjects.

Recently Prange Hansen (38) reported that the concentration of plasma GH during exercise increased to considerably higher levels in untreated diabetic patients than in non-diabetics. In the present study only one of the six diabetics showed this abnormal response. On the other hand the basal levels of GH were significantly higher in the diabetics than in other subjects. Although GH may not play an essential role in lipid mobilization in non-diabetic subjects during short term exercise (21 27 28 29 40) the consistently high GH levels of the insulin-dependent diabetics might facilitate lipolysis during muscular work

Some authors have reported that early and/or augmented rises in the concentration of GH after oral glucose (46, 53), i.v. glucose (4) and i.v. tolbutamide (4) are observed more frequently among the offspring of diabetic parents than in controls. In earlier studies from this laboratory plasma GH in prediabetics during glucose infusions (15) and insulin hypoglycemia (16) was found to be normal.

In the present study an augmented rise in the concentration of GH was seen only in pre diabetics and in one of the diabetics in clinical remission. These hyperresponsive prediabetics also showed increased lipid mobilization. On the other hand the diabetic patient in clinical remission and the other prediabetic subjects who demonstrated a diabetic-like pattern in the lipid response to exercise showed normal plasma GH curves. This may suggest that there is probably no causal relationship between the current concentration of plasma GH and the rise in the FFA and glycerol levels.

Decreased insulin output is the common denominator of all phases of diabetes, including prediabetes (13, 14). We have no explanation of the finding that only 4 out of 10 of the prediabetics in the present investigation behaved like diabetics with respect to lipolysis during exercise. We have previously demonstrated that 15–20 of normal subjects with normal glucose tolerance are prediabetics. Obviously not more than about 5 of these can eventually develop a decreased glucose tolerance. It is a challenging idea that the above 4 prediabetics represent those who may later become diabetic.

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (B70-19 34-06B, B71-19 34-07C, B71-19 1034-05A), Knut and Alice Wallenberg's Foundation and the Swedish Diabetes Association.

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Acta Med. Scand. Vol. 193 pp. 23–33 1973

MILD DIABETES IN YOUNG SUBJECTS

Clinical Aspects and Plasma Insulin Response Pattern

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Abstract. The clinical aspects and the plasma insulin response patterns in young subjects with mild diabetes are described. Young subjects with mild diabetes have no or very few symptoms and can be controlled by diet or oral antidiabetic drugs. The mild diabetics are divided into two groups according to the fasting blood glucose concentration. Subjects having *normal* fasting blood glucose concentration but diabetic glucose tolerance test are called *juvenile glucose tolerance test diabetics* and those with *elevated* fasting blood glucose concentration and diabetic glucose tolerance test are designated *mild juvenile diabetics*.

It is demonstrated that juvenile glucose tolerance test diabetics have normal plasma insulin response after i glucose and tolbutamide. The plasma insulin response to oral glucose, however, is weak and slow in comparison to non-diabetics. In contradistinction, mild juvenile diabetics exhibit weak and delayed plasma insulin response both to i and oral glucose and to i. tolbutamide. In comparison with classic juvenile diabetics, juvenile glucose tolerance test diabetics and mild juvenile diabetics have much higher plasma insulin level during the tests.

Diabetes mellitus in children and young adults usually sets in abruptly within days or weeks. Severe symptoms including thirst, polyuria, weight loss, general malaise and sometimes paraesthesia, muscle cramps and blurred vision appear. Ordinary laboratory investigations disclose severe carbohydrate intolerance with a pronounced tendency to ketoacidosis. This is the *classic juvenile diabetes*.

Previously it was generally accepted that mild diabetes of maturity-onset type did not occur or at least was extremely rare in children and young adults. In 1960 Fajans and Conn (5) reported that mild diabetes can be recognized in children and young adults and this has been confirmed in later communications (1 16). The plasma insulin response pattern in this peculiar type of diabetes has previously been described in two preliminary reports (11 12).

Mild diabetes has been found in both obese and non-obese young people and the degree of carbohydrate intolerance has varied. Patients with normal fasting blood sugar and minor abnormalities of the glucose tolerance test, as well as patients with both elevated fasting and elevated blood sugar levels throughout the day have been studied.

The main topic of this communication is a presentation of the plasma insulin response patterns in *non-obese* patients with *mild juvenile diabet* and in *non-obese* patients with an even milder degree of carbohydrate intolerance, i.e. *juvenile glucose tolerance test diabetes*.

We use the term mild juvenile diabetes to designate a type of diabetes found in normal weight children and young adults with elevated fasting blood sugar levels as well as elevated blood sugar levels throughout the day with no or only very mild symptoms and no ketonuria. This type of diabetes can be controlled by carbohydrate restriction or oral antidiabetic drugs.

We use the term juvenile glucose tolerance test diabetes to designate the type of diabetes occurring in young subjects of normal weight characterized by a normal fasting blood sugar level but a diabetic glucose tolerance test. The patients have no symptoms, only mild glucosuria and no ketonuria. The diabetes can most often be controlled by carbohydrate restriction.

The plasma insulin patterns of these two groups will be described and compared mutually

Table I. *Clinical data of the juvenile glucose tolerance test diabetics*

Case no	Sex	Age (y.)	Height (cm)	Weight (kg)	of average b.wt.	Family history	Diagnosis
1	♂	4	182 182	70 63	94 85		Routine examination during military duty showed glucosuria in 1965
2	♂	1	150 175	4 58	110 91		Routine examination by the school doctor showed glucosuria in 1964
3		31	158 158	44 44	77 77		Elevated blood sugar during dmission because of migraine Sept. 67
4		1	149	39	98		Routine examination by the school doctor showed glucosuria May 67
5		4	158 158	54 51	103 101	Mother insulin-treated diabetes since the ge of 38	Glucosuria during 5th–6th mo. of pregnancy Sept. 67
6	♂	17	169 169	6_ 64	100 101	Mother insulin-treated diabetes since the age of 14 Fh of the mother's sisters insulin-treated diabetes. Two cousins insulin-treated diabetes	Glucosuria discovered during admission for tonsillitis in 1967
7	♂	6	132	?7	94	Grandmother diabetes in old ge	Routine prophylactic children examination showed glucosuria in 1967

d with the insulin response patterns of non-iabetics and patients with classic ju enile diabetes

METHODS

The insulin response patterns of the mild diabetics (juvenile glucose tolerance test diabetics and mild juvenile diabetics) were compared ith those of 1_ non-diabetics (5 females, 7 males) whose ages ranged from 18 to 3? years (mean 25.6 ± 1.3) and weights from 71 to 108% of the average b.wts. (mean 92.8 ± _.9) (Documenta Geigy Scientific Tables, 6th ed., p. 623). They were also compared ith those of 14 classic juvenile diabetics (4 females, 10 males) whose weights ranged from 67 to 103% of the average b.wts. (mean 85.4 ± _.8) and ages from 11 to 33 years (mean 22.6 ± _.0)

In our definition *normal glucose tolerance test* means fasting blood glucose concentration below 100 mg/100 ml and blood glucose falling to below 120 mg/100 ml within _.5 hours after 100 g glucose by mouth. *Diabetic glucose tolerance test* is defined as a glucose tolerance test where the blood glucose concentration has not fallen below 120 mg/100 ml within 2.5 hours after 100 g glucose by mouth, i.e. no borderline group is included in our definition. Patients hose blood glucose concentration has not fallen to below 120 mg/100 ml within _.5 hours were called *diabetics*. None of our patients had signs or symptoms of pituitary, adrenal or diffuse pancreatic diseases.

The following schedule was used.

Days 1 and 3. All the subject were on standardized d et containing 300 g carbohydrate and 2 000 calories per day. On the _nd day ear blood sugar was measured every hour during the daytime and every second hour during the night.

Day 4. I glucose tolerance test. Glucose, 25 g (0 solution), was injected into an antecubital vein in the course of 4 min. After 2 min of injection stopwatch started and blood was dra n every 10 min fo 1 hour. The results of the i. glucose tolerance tests ere expressed, inter alia, by the *k*-value calculated according to Conard (4).

Day Single oral glucose tolerance test. Glucose, 100 g (33% solution), flavoured ith lemon as ingested in 3–4 min after drawing the fasting sample.

Day 6: Double oral glucose tolerance test. Glucose, 100 g (33% solution), flavoured ith lemon was ingested in 3–4 min after drawing the fasting blood sample and the same load was given 1 hour later.

Day 7. Intermission.

Day 8. I tolbutamide test. Sodium tolbutamide 1 g (5% solution, Rastinon®), was injected in the course f 2 min. After 1 min of injection a stopwatch was started and blood as drawn every 10 min for 1 hour.

During the oral tests blood was collected every 15 m in the first hour and every 30 min in the last 2 hours.

Blood sugar range (mg%)	Glucosuria (%)	Tests performed	Treatment
90–190	0.2–0.3	June 67	June 67 free diet
200–450	5–7 Ketonuria	Dec. 69	Dec. 69 NPH 7
80–160	0.3–1	Aug. 67	Aug. 67 free diet
105–160	1–2	Sept. 71	Sept. 71 free diet
110–320	0.4–0.7	Oct. 67	Oct. 67 free diet – sugar
115–260	0.2–0.3	Oct. 68	Oct. 68 free diet – sugar
80–150		Aug. 67	Aug. 67 free diet
90–180	0.5	March 68	March 68 free diet
80–190	0.3	Aug. 68	Aug. 68 free diet
85–175	0.6–1.2	May 69	May 69 free diet – sugar
100–230	3	Oct. 70	Oct. 70 free diet – sugar
90–145		May 69	May 69 free diet

All tests were made in the ward, but the patients were up and about and not bed-resting except during the night. The tests were performed in the morning after 12 to 14 hours' fast (water was allowed according to thirst) and with the subjects in recumbency.

Antecubital venous blood was obtained from an indwelling plastic catheter (length 15 cm). Whole blood glucose was determined by the glucose oxidase method (3) in single determinations. Plasma insulin was determined in triplicate by a slight modification of the radioimmunoassay of Hales and Randle (8) after addition of EDTA. The blood samples were centrifuged and stored at –20°C until analysis. The diurnal blood sugar taken on the 2nd day on diet was estimated by an o-toluidine method (7).

Statistical calculations were carried out with the non-parametric Wilcoxon test (17) and Page significance test for linear ranks (15). The 5% limit was accepted as an indication of significance.

RESULTS

Clinical Aspects of Mild Diabetes in Young Subjects

Juvenile glucose tolerance test diabetes

The pertinent clinical data of the three females and four males with juvenile glucose tolerance



Fig. 1. Individual diurnal blood sugar curves of the juvenile glucose tolerance test diabetics. Arrows indicate meals.

test diabetes are given in Table I. Their ages ranged between 6 and 31 years and the percentages of average b.wts. between 77 and 110 at the time of diagnosis. Three of the patients had a family history of diabetes mellitus. None had symptoms of diabetes mellitus and all were discovered accidentally by the finding of glucosuria. Their mildly elevated diurnal blood sugar levels are shown in Fig. 1. All the patients had a normal fasting blood glucose level (mean of fasting blood glucose concentrations before the four tolerance tests < 100 mg/100 ml). None of them had ketosis: they had no or only mild glucosuria. One of the patients (case 1) has developed classic juvenile diabetes with ketosis and no insulin response to glucose or tolbutamide and has been put on insulin therapy (19). The six other patients are, however, still controlled on free diet or free diet minus sugar.

Mild juvenile diabetes

Table II shows the pertinent clinical data of the four females and five males with mild juvenile diabetes. Their ages ranged between 14 and 31 years and the percentages of average b.wts. be-



Fig. 2. Individual diurnal blood sugar curves of the mild juvenile diabetics. Arrows indicate meals.

Table II. *Clinical data of the mild juvenile diabetics*

Case no	Sex	Age (y)	Height (cm)	Weight (kg)	% of average b.wt.	Family history	Diagnosis
1	♂	14	165 171	48 51	92 88	Mother insulin-treated diabetes since the age of 25	Routine examination by the school doctor revealed glucosuria Nov. 65
2	♀	18	166 168	53 55	94 94		Routine examination by the school doctor revealed glucosuria Sept. 60
3	♀	20	169 169	64 74	108 125	Mother and father insulin-treated diabetes since adolescence and middle age	Routine examination by the school doctor revealed glucosuria in 1961
4	♀	24	159 159	62 72	114 131		Perhaps thirst for 2-3 years. Glucosuria during 5th mo. of pregnancy July 67 Delivery Oct. 67
5	♂	14	164 172	5. 55	105 88	Cousin and mother' cousin diabetes	Tiredness for 2 3 mo. Routine examination by the school doctor revealed glucosuria Sept. 67
6	♂	21	176 176 176	61 58 54	87 83 77	Father tablet and brother insulin-treated diabetes since the ages of 59 and 15	Glucosuria discovered Oct. 68 in connexion with taking out a lif insurance
7	♂	30	171 171	62 65	86 90	?	Routine examination revealed glucosuria Aug. 67
	♀	31	169 169	69 66	106 103		Glucosuria found during pregnancy in 1966
	♂	30	176	74	97	Two aunts diabetes in middle age	In May 70 had had thirst, polyuria and weight loss for 1 year

Ten days after delivery

tween 86 and 114 at the time of diagnosis. Five of the patients had a family history of diabetes. Three patients (cases 4, 5 and 9) had mild symptoms of diabetes 2–3 months to 2–3 years before the diagnosis. The disease was discovered accidentally (except case 9) by the finding of glucosuria at routine examination. Their moderately elevated diurnal blood sugar levels are seen in Fig. 2. All had elevated fasting blood glucose levels (mean of fasting blood glucose concentrations before the four tolerance tests between 100 and 200 mg/100 ml). Until now three of the nine patients have developed classic juvenile diabetes with ketosis and no plasma insulin response to glucose. They have been put on insulin therapy. This happened after 2 to 2 1/2 years of known mild juvenile diabetes in two 16-year-old boys (cases 1 and 5) and in one 23-year-old male (case 6). The condition has, however, remained unchanged in six of the patients for 10, 10, 4, 4, 5 and 2 years, respectively, and their diabetes is controlled by diet plus tolbutamide and/or phenformin.

Juvenile Glucose Tolerance Test Diabetes

Intravenous tests

Blood glucose. The mean blood glucose curves of the juvenile glucose tolerance test diabetics and the non-diabetics were not very different during the two i.v. tests (Fig. 3), but they were very much lower than those of the classic juvenile diabetics. The mean *k* value of the juvenile glucose tolerance test diabetics (1.46 ± 0.88) was not

Blood sugar range (mg %)	Glucosuria (%)	Test performed	Treatment
90–230	0.3	May 66	July 68 phenformin 50 mg 4
170–420	4–7 ketonuria	June 68	Oct. 70 NPH 7 (morning) + NPH 5 (evening)
170–220	1–4	June 66	June 66 free diet
100–210	0.2–0.3	Feb. 68	Feb. 68 tolbutamide 500 mg 1
200–350	5–6	Oct. 66	Oct. 66 tolbutamide 500 mg 2
140–260	6	Jan. 68	Jan. 68 tolbutamide 500 mg 3
140–230	0.4–0.7	Oct. 67*	Oct. 67 carbohydr. fixed diet
140–340	3–5	Jan. 69	Jan. 69 tolbutamide 500 mg 3
170–240	3–5	Nov. 67	Dec. 67 tolbutamide 500 mg 3 + phenformin 50 mg 3
140–240	0.4–0.7 ketonuria		Jan. 70 NPH 7 + Reg. 3 (morning)
150–300	3–6	Dec. 68	Dec. 68 carbohydr. fixed diet
140–320	2–4	June 69	June 69 tolbutamide 500 mg 3 + phenformin 50 mg 2
230–310	7 ketonuria	Feb. 71	Feb. 71 NPH 5 (morning)
170–280	0.5–2	Jan. 70	Jan. 70 carbohydr. fixed diet
240–380	3–4	Aug. 70	Aug. 70 tolbutamide 500 mg 3
110–190	0.3–0.6	Feb. 70	Feb. 70 free diet
120–220	0.2	May 70	May 70 free diet
130–270	1–3	May 70	June 70 carbohydr. fixed diet

significantly different from that of the non-diabetics (1.26 ± 0.12) but significantly higher ($p < 0.01$) than that of the classic juvenile diabetics (0.52 ± 0.05).

Plasma insulin. The plasma insulin responses in the juvenile glucose tolerance test diabetics and the non-diabetics were similar during the two i.v. tests (Fig. 3). Both groups showed a brisk rise in plasma insulin concentration after both stimuli, the highest value observed was the 10-min value and the plasma insulin concentration subsequently fell slowly towards the fasting level. In contradistinction classic juvenile diabetics showed no rise at all in plasma insulin to any of the stimuli.

Oral tests

Blood glucose. The mean blood glucose curves of the juvenile glucose tolerance test diabetics did not differ from those of the non-diabetics in the first $^1/_2$ to $^3/_4$ hour but were higher in the last 2 hours during both oral tests (Fig. 4). The mean blood glucose curves of the classic juvenile diabetics were very much higher than those of the juvenile glucose tolerance test diabetics and the non-diabetics at all times during both tests.

Plasma insulin. Patients with juvenile glucose tolerance test diabetes showed a rise in plasma insulin during both tests (Fig. 4). Compared to non-diabetics, however, the rise was weak and retarded. The highest value during the single oral test of the non-diabetics—the 1-hour value—was significantly higher than the 1 hour value of the juvenile glucose tolerance test diabetics ($p < 0.05$). During the double oral glucose tolerance test the highest value of the non-diabetics—the $1^1/_2$-hour value—was not different from the $1^1/_2$-hour value of the juvenile glucose tolerance test

Table II. *Clinical data of the mild juvenile diabetics*

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3	♀	20	169 169	64 74	108 125	Mother and father insulin-treated diabetes since adolescence and middle age	Routine examination by the school doctor revealed glucosuria in 1961
4	♀	[illegible]4	159 159	62 72	114 131		Perhaps thirst for 2–3 years. Glucosuria during 5th mo. of pregnancy July 67 Delivery Oct. 67
5	♂	14	164 17[illegible]	52 55	105 88	Cousin and mother's cousin diabetes	Tiredness for 2 3 mo. Routine examination by the school doctor revealed glucosuria Sept. 67
6	♂	1	176 176 176	61 58 54	87 83 77	Father tablet- and brother insulin-treated diabetes since the ages of 59 and 15	Glucosuria discovered Oct. 68 in connexion with taking out life insurance
7	♂	30	171 171	62 65	86 90	?	Routine examination revealed glucosuria Aug. 67
		31	169 169	69 66	106 103		Glucosuria found during pregnancy in 1966
	♂	30	1[illegible]6	74	97	Tw[illegible] aunts diabetes in middle age	In May 70 had had thirst, polyuria and weight loss for 1 year

Ten days after delivery

tween 86 and 114 at the time of diagnosis. Five of the patients had a family history of diabetes. Three patients (cases 4 5 and 9) had mild symptoms of diabetes [illegible]–3 months to –3 years before the diagnosis. The disease was discovered accidentally (except case 9) by the finding of glucosuria at routine examination. Their moderately elevated diurnal blood sugar levels are seen in Fig. 2. All had elevated fasting blood glucose levels (mean of fasting blood glucose concentrations before the four tolerance tests between 100 and 200 mg/100 ml). Uptil now three of the nine patients have developed classic juvenile diabetes with ketosis and no plasma insulin response to glucose. They have been put on insulin therapy This happened after 2 to 2 [illegible]/2 years of known mild juvenile diabetes in two 16-year-old boys (cases 1 and 5) and in one [illegible]3-year-old male (case 6) The condition has, however remained unchanged in six of the patients for 10, 10 4 4 5 and 2 years, respectively and their diabetes is controlled by diet plus tolbutamide and/or phenformin.

Juvenile Glucose Tolerance Test Diabetes

Intravenous tests

Blood glucose The mean blood glucose curves of the juvenile glucose tolerance test diabetics and the non-diabetics were not very different during the two i.v. tests (Fig. 3) but they were very much lower than those of the classic juvenile diabetics. The mean *k* value of the juvenile glucose tolerance test diabetics (1.46 ± 0.88) was not

Table III. *Page's test used on the plasma insulin concentrations ($\mu U/ml$) during the double oral glucose load in classic juvenile diabetics*

	Hours –																		
Ranking	0		1/4		1/2		3/4		1		1½		2		2½		3		
Case no.	(1)		(2)		(3)		(4)		(5)		(6)		(7)		(8)		(9)		$= Y$
1	24	*1*	30	*7*	25	*2*	26	*5*	29	*6*	40	*9*	33	*8*	25	*3*	25	*4*	
2	18	*7*	17	*4*	6	*1*	13	*3*	20	*8*	18	*6*	18	*5*	12	*2*	20	*9*	
3	8	*6*	5	*1*	7	*3*	8	*5*	11	*7*	8	*4*	12	*9*	11	*8*	6	*2*	
4	24	*5*	30	*9*	23	*4*	20	*1*	24	*6*	22	*3*	26	*8*	26	*7*	22	*2*	
5	12	*1*	18	*4*	12	*2*	18	*3*	22	*5*	22	*6*	34	*9*	28	*8*	24	*7*	
6	4	*7*	3	*3*	2	*2*	3	*4*	5	*9*	5	*8*	1	*1*	3	*5*	6	*6*	
7	0	*1*	5	*3*	9	*4*	10	*5*	4	*2*	16	*8*	16	*9*	14	*7*	12	*6*	
8	3	*4*	2	*3*	6	*7*	0	*2*	0	*1*	8	*8*	8	*9*	5	*6*	4	*5*	
9	4	*2*	2	*1*	5	*6*	4	*3*	4	*4*	4	*5*	6	*7*	7	*8*	10	*9*	
10	5	*6*	9	*9*	4	*3*	3	*2*	3	*1*	6	*7*	8	*8*	5	*5*	4	*4*	
$\Sigma^m X_{ij}$	=	*40*		*44*		*34*		*33*		*49*		*64*		*73*		*59*		*54*	$450 = \Sigma^n \Sigma^m X_{ij}$
$Y_j \Sigma^m X_{ij}$	=	*40*		*88*		*102*		*132*		*245*		*384*		*511*		*472*		*486*	$2\,460 = \Sigma^n (Y_j \Sigma^m X_{ij}) = L$

The definition of the statistic is $L = \Sigma^n (Y_j \Sigma^m X_{ij})$, where Y_j is the hypothetical ranking of the *j*th column and $\Sigma^m X_{ij}$ equals the sum of ranks observed in the *j*th column. The statistic may be defined in words as the sum of products of the predicted ranking of each column times the sum of observed ranks of the column. Cases 11 and 12 were excluded from the calculations because some of the patients' figures were lacking.

Figs. 7 and 8 show the results of follow-up tests in five cases (nos. 2, 3, 6, 7 and 8) who have not progressed to insulin-requiring diabetics. The follow-up study was performed 1 $^{1}/_{4}$–2 years after the initial study and after the patients have been off oral antidiabetic drugs for three days. It is evident that the mean glucose and plasma insulin responses are unaltered from the initial to the follow-up tests.

Comparison of the Plasma Insulin Response Pattern of the Juvenile Glucose Tolerance Test Diabetics and the Mild Juvenile Diabetics

Intravenous tests

Blood glucose. The mean blood glucose curves of the juvenile glucose tolerance test diabetics were higher than those of the mild juvenile diabetics during both tests (Fig. 9). The mean *k* value of the juvenile glucose tolerance test diabetics (1.46 ± 0.88) was significantly higher ($p < 0.01$) than that of the mild juvenile diabetics (0.59 ± 0.07).

Plasma insulin. The patients with juvenile glucose tolerance test diabetes exhibited a normal brisk rise in plasma insulin after both stimuli in comparison with patients with mild juvenile diabetes, who showed a weak and slow rise after the same stimuli (Fig. 9). The highest value during both tests—the 10-min value—of the juvenile glucose tolerance test diabetics was not significantly different from that of the mild juvenile diabetics.

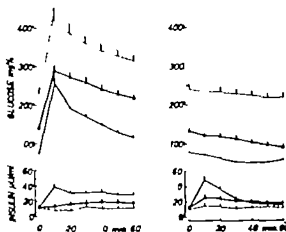


Fig. 5. Glucose and plasma insulin responses to i. glucose (left) and i. tolbutamide (right) in mild juvenile diabetics (●—●), classic juvenile diabetics (-·-·-) and non-diabetics (—). Mean ± S.E.M.

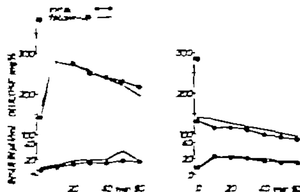


Fig. 7 Glucose and plasma insulin responses to i.v. glucose (left) and i.v. tolbutamide (right) during initial (●—●) and follow-up tests (—). Mean curves.

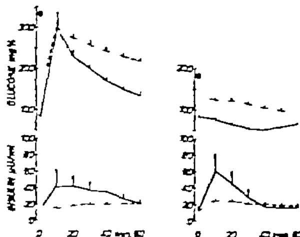


Fig. 9 Glucose and plasma insulin responses to i.v. glucose (left) and i.v. tolbutamide (right) in juvenile glucose tolerance test diabetes (—) and mild juvenile diabetes (- -). Mean ± S.E.M.

rare conditions. All we can say is that in a study of the glucose tolerance and plasma insulin and growth hormone response pattern of 320 schoolchildren in a Danish parish school we found no case of mild diabetes (13).

The natural history of mild diabetes in young subjects is not well known. We know, however, that in some cases it represents an early phase of classic juvenile diabetes. Thus one of the [illegible] glucose tolerance test diabetics and three [illegible] the mild juvenile diabetics have developed classic juvenile diabetes with severe symptoms and [illegible]-acidosis and have no longer any plasma insulin response to either glucose or tolbutamide. They have now been put on insulin treatment. In the majority of the subjects the mild diabetic state has remained unchanged and may be an expression of the kind of metabolic disturbance usually present in old people with maturity-onset diabetes.

The purpose of the present study was to examine the plasma insulin response pattern in mild diabetes in children and young subjects. We found that patients with juvenile glucose tolerance test diabetes have a normal plasma insulin re

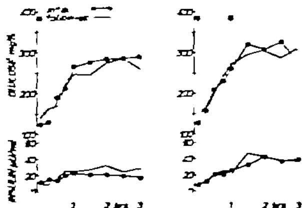


Fig. 8. Glucose and plasma insulin responses to [illegible] single (left) and double (right) oral glucose load during initial (●—●) and follow-up tests (—). Mean curves.

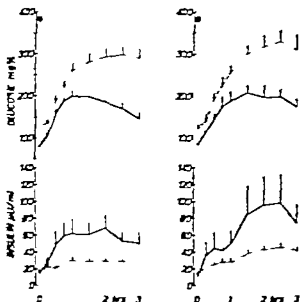


Fig. 10 Glucose and plasma insulin responses to a single (left) and double (right) oral glucose load in juvenile glucose tolerance test diabetes (—) and mild juvenile diabetes (- -). Mean ± S.E.M.

sponse to i.v. glucose and tolbutamide. Their plasma insulin response to oral glucose is, however, weak and slow in comparison with non-diabetics. In contradistinction, mild juvenile diabetics exhibit a weak and delayed plasma insulin response to both i.v. and oral glucose and to i.v. tolbutamide. Both the juvenile glucose tolerance test diabetics and the mild juvenile diabetics show a much higher plasma insulin response during all four tests than the classic juvenile diabetics.

There are only few reports about the plasma insulin response pattern in non-obese young subjects with mild diabetes. In 1967 the first reports appeared in two preliminary communications (11, 12). In 1969 Chiumello et al. (2) showed that non-obese children with glucose tolerance test diabetes had a slow and weak plasma insulin response to oral glucose compared to normal controls. Their results are thus in agreement with ours. In an extensive and thorough study Fajans et al. (6) confirmed our preliminary and the present findings that young non-obese subjects with mild diabetes have a delayed and subnormal insulin response to oral glucose. Murthy et al. (14) failed, however, to find any significant difference between the magnitude of the plasma insulin response of children with "chemical" diabetes and controls after i.v. glucose and i.v. tolbutamide. This may easily be explained by the criteria used in the study for the diagnosis of chemical diabetes, which—as they state—may have allowed the inclusion of some normal children.

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THE EFFECT OF LONG TERM ADMINISTRATION ON THE ABSORPTION OF METHYLSCOPOLAMINE IN MAN

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Abstract. ^{14}C-methylscopolamine (15 mg) and the non-absorbable marker polyethylene glycol (PEG) have been given orally to six healthy subjects who had received oral doses of 10–25 mg of the drug for 10–14 days prior to the experiments. With this treatment the subjects experienced dryness in the mouth. Compared to untreated subjects studied previously in the same way, the passage of PEG through the small intestine appeared to be retarded. The absorption of the labelled compound—as evidenced by the concentration of radioactivity per unit marker in gastrointestinal aspirates, the blood levels, and the urinary faecal and biliary excretion of label—was the same whether the subjects were pretreated with methylscopolamine or not.

In recent studies the quaternary nitrogen compounds methylscopolamine (1), methylatropine (2), butylscopolamine (5) and Acabel (3) were poorly absorbed following oral administration. The uptake of a single dose of methylscopolamine was only 20% or less. Since anticholinergic drugs may influence gastric emptying and intestinal peristalsis, their absorption may be different during long-term treatment. This possibility was considered in the present study on the uptake of methylscopolamine in healthy subjects pretreated with the drug.

EXPERIMENTAL PROTOCOL

Non-labelled methylscopolamine was given orally to six subjects for 10–14 days prior to the experiment. The original dose of 5 mg every 12 hours was increased by 5 mg every second day until the subjects experienced an almost constant dryness in the mouth. The effect required was obtained with 10–25 mg methylscopolamine/day. The last dose was administered 2–3 hours before the intake of the test solution.

All experiments started in the morning, the subjects having fasted over night. One or several days before the experiment the subject was provided with an intestinal double lumen tube. This was inserted through the nose and allowed to pass to the desired level in the intestine. The position of the tube was controlled by X-ray. A gastric tube was inserted in some subjects before the start of the experiment.

^{14}C-methylscopolamine (15–20 μCi) and 5 g polyethylene glycol (PEG, non-absorbable marker) were dissolved in 50 ml water and administered orally to the subjects (Table I). Gastrointestinal aspirates were drawn at various intervals and analyzed for radioactivity and PEG. Concentrated samples of duodenal bile were obtained after injection of 37.5 Ivy units of cholecystokinin (Table I). Blood samples were drawn at 15, 30, 45, 60 and 90 min and at 2, 3, 4, 7, 10 and 24 hours. Urine and faeces were collected for 7 days.

MATERIAL AND METHODS

Subjects. Male, healthy transport workers, 35–61 years old, volunteered for the investigation.

Material. Labelled and unlabelled methylscopolamine included in the test solution were obtained from Pharmacia, Uppsala, Sweden. ^{14}C-methylscopolamine (25 μCi/mg) was synthesized by methylation of scopolamine with ^{14}C-methyliodide. The radiochemical purity of the label (> 98%) was ascertained by thin layer chromatography (TLC) on silica gel plates as described earlier (1). Cholecystokinin was obtained from the Gastrointestinal Hormone Research Group, Chemistry Department, Karolinska Institutet, Stockholm. PEG (mol. wt. 4 000) was purchased from Kebo, Stockholm, Sweden.

Determination of radioactivity. The radioactivity was determined with a liquid scintillator (Packard model 3003). Plasma samples (1 ml) were dissolved in 15 ml of an emulsifier (Insta-Gel, Packard). Aliquots of gastrointestinal aspirates (0.1 ml) and urine (1 ml) were pipetted into 15 ml of the scintillation liquid described by Bray (4). Faeces were homogenized and lyophilized. Aliquots of the dried powder were analyzed for radioactivity using a combustion technique modified from Schöniger (10). The recovery of radioactivity added to faeces homogenates ranged between 95.6 and 99.6% (mean 97.9). Quenching was corrected for by internal standardization.

Table I *Protocol of experiments*

Subject no.	Collection of urine and faeces (d.)	Site of gastrointestinal aspiration. Distance from the nose (cm)	Injection of cholecystokinin. Time after administration of label (h)
1	7	50 80, 110	4, 7
2	7	50, 80, 110	4
3	7	80, 110	4 7
4	7	80, 110	4, 7
5	7	80, 110	
6	7	110	

Determination of the absorption of radioactivity The uptake of label was calculated by measuring the ratio between the radioactivity per mg PEG of series of aspirates and that of the test solution (A/T ratio). The ratios were calculated only for aspirates in which the concentration of PEG exceeded 1 mg/ml. PEG was analyzed as described by Hydén (7).

RESULTS

Absorption of radioactivity from the stomach and the upper small intestine

According to the A/T ratios there was no absorption of radioactivity when PEG and the label were passing the stomach (Table II). Such ratios recorded for duodenal aspirates obtained 80 cm the nose demonstrated that up to 15% of label had been taken up. The cumulative absorption as evidenced by aspirates from the upper jejunum (110 cm from the nose) ranged between 11 and 29% (mean 20). The results of one experiment are illustrated in Fig. 1

Table II. *A/T ratios of gastrointestinal aspirates*

Subject no.	Level (cm)[a]	Time (min)[b]	No. of samples	Mean A/T ratio[c]
	Collection of aspirates			
1	50	5–30	6	1.01 (0.91 1.11)
2	50	5–100	10	0.99 (0.77 1.10)
1	80	5–45	9	0.96 (0.75–1.12)
2	80	5–130	9	0.98 (0.92 1.03)
3	80	3–42	9	0.93 (0.81–0.99)
4	80	5–50	8	0.93 (0.79–1.08)
5	80	9–97	12	0.85 (0.71–0.94)
1	110	11–88	15	0.71 (0.40–0.93)
2	110	8–165	12	0.89 (0.69–1.05)
3	110	13–42	5	0.85 (0.71–0.98)
4	110	6–247	23	0.79 (0.50–0.98)
5	110	9–138	10	0.77 (0.71–0.86)
6	110	5–100	12	0.80 (0.65–1.01)

[a] Distance from the nose.
[b] After administration of the test solution.
[c] Ranges given within parentheses.

Plasma levels of label

In most cases the plasma showed detectable amounts of radioactivity from 30 min to 7 hours after the intake of the test solution. In subjects 2–6 the peak level was observed at 90–180 min, when the total plasma volume contained 0.3–0.8% of the dose given. The highest concentration, 1.7% of administered radioactivity per plasma volume, was observed at 90 min in subject 1

Excretion of radioactivity in urine and faeces

The total excretion of label in the urine and faeces during 7 days averaged 87.9% and was close to 100% in two subjects. The cumulative recovery in the urine ranged between 8.1 and 18.2% (mean 13.2) The major part (mean 11.3%) was recovered within 12 hours.

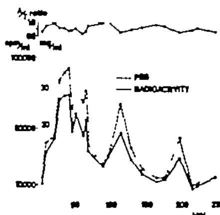


Fig. 1 Concentration of radioactivity (cpm/ml) and PEG (mg/ml) and A/T ratios in specimens of jejunal aspirates. Subject 4.

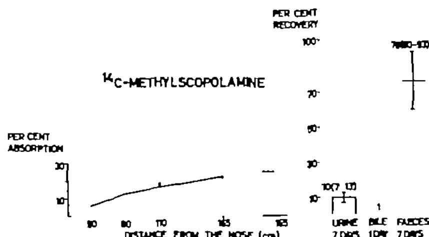


Fig. 2. The A/T ratios of gastrointestinal aspirates and the urinary biliary and faecal excretions of label after oral administration of ^{14}C-methylscopolamine in untreated subjects. Means (and range limits)

Biliary excretion of radioactivity

Small concentrations of radioactivity (122–1 073 cpm/ml) were observed in the duodenal aspirates obtained at 4 hours. When cholecystokinin was injected, the concentration of bilirubin increased markedly but that of the label only slightly. At 7 hours, the administration of cholecystokinin appeared to have almost no effect on the small concentration of radioactivity recorded for the duodenal aspirates.

DISCUSSION

The absorption of a compound after oral administration may be regulated by specialized, saturable processes. It may also depend upon the time during which the compound is in contact with the sites of intestinal absorption. Thus, riboflavin was taken up more efficiently when administered with food, a phenomenon related to a prolongation of the time of residence within the proximal part of the small intestine (8).

Mózsik et al. (9) administered the anticholinergic compound oxyphencyclimine, a tertiary amine, and measured the urinary excretion of the intact drug by bioassay technique. The values encountered after long-term administration were almost twice as high as those observed after single doses. Such differences were not observed after i.m. injections of the drug and the authors sug-

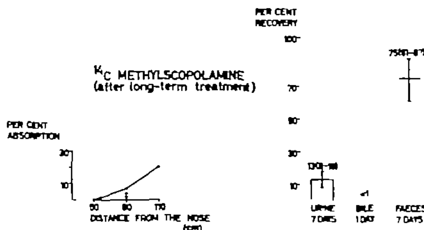


Fig. 3. The A/T ratios of gastrointestinal aspirates and the urinary biliary and faecal excretions of label after oral administration of ^{14}C-methylscopolamine after long-term treatment. Means (and range limits).

gested that long-term treatment with oxyphencyclimine had a favourable effect on the gastrointestinal absorption of the drug itself. Holtz et al. (6) studied the salivary excretion in healthy subjects and reported that the efficacy of oral doses of methylscopolamine increased progressively following repeated administration of the drug (5 mg every 12 hours).

The aim of the present investigation was to study whether long-term administration of methylscopolamine could influence the absorption of the drug. As an effect of the treatment all subjects experienced dryness in the mouth for more than one week prior to the experiments. During the experiments they also appeared to have a retarded passage through the small intestine. In aspirates collected 110 cm from the nose in two out of three untreated subjects, the concentration of PEG was less than 1 mg/ml after only 20 min (1). Such low concentrations in the present subjects were not reached until after 88 min in five out of six cases (Table II).

The A/T ratios demonstrate that approximately 20% of the radioactivity was absorbed in the duodenum and the proximal part of the jejunum. Since all label in the aspirates appeared to be attached to methylscopolamine (1) it is conceivable that the uptake of radioactivity reflected the absorption of the intact drug. As evidenced by the urinary excretion of 8–18% of the administered label and the very small biliary output of radioactivity, the main uptake of methylscopolamine was confined to the very proximal part of the small intestine.

The present data concerning A/T ratios, blood levels, urinary, faecal and biliary excretion of label are quite similar to those obtained in the previous study of untreated subjects (Figs. 2 and 3). It appears, then, that the uptake of methylscopolamine does not change upon long-term administration and that the phenomenon reported by Holtz et al. (6) should be related to other factor(s) than changes in the intestinal absorption of the drug.

ACKNOWLEDGEMENTS

The present investigation was supported by the Swedish Medical Research Council and AB Pharmacia, Uppsala, Sweden.

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Acta med. scand. Vol. 193 pp. 39–44 1973

ATRIAL FIBRILLATION IN ACUTE MYOCARDIAL INFARCTION

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Abstract. Atrial fibrillation (AF) observed during the first 24 hours in a coronary care unit (CCU) has been analysed in 450 consecutive patients with acute myocardial infarction. AF was seen in 15% of the patients and was found to be associated with a significantly raised hospital mortality 38% against 18% for the remainder. Repeated episodes of AF were not associated with a raised mortality. The patients with AF were older, more often in left heart failure, and more commonly lacked diagnostic ECG findings of an acute infarction. Sinus rhythm was present at the time of discharge from the CCU, or prior to death in the CCU, in 67% of the patients with AF.

Continuous ECG monitoring of patients during the early phases of acute myocardial infarction (AMI) has resulted in a much improved knowledge of the complicating arrhythmias.

A recent study of the various arrhythmias seen in our coronary care unit (CCU) in 450 consecutive admissions has revealed an increased mortality in patients with atrial fibrillation (AF) (1). As this finding differs from several other reports (2, 3, 4, 7, 11) a further analysis was felt to be necessary. This study therefore deals with the incidence, mortality and certain other aspects of AF as seen in 450 consecutive patients admitted in 1968–69.

METHODS

Serafimerlasarettet serves an undefined population within Greater Stockholm and has about 200 beds for general medicine. Acute admissions are received in the Casualty Department, from where the CCU is immediately contacted following the admission of a patient who after a rapid and superficial appraisal might fulfil at least one of the following admission criteria.

1) Central chest pain lasting for more than 15 min beginning within the last 48 hours. 2) Frank pulmonary oedema without previously known valvular lesion or uraemia. 3) Shock without suspicion of acute hypovolaemia or intoxication. 4) Syncope with ECG evidence of AMI. 5) Intractable angina pectoris. The last two criteria were added on Sept. 18, 1968.

The general policy of this CCU and the criteria employed have been previously described (8). In the present study an analysis of AF during the first 24 hours was performed, as this is the period of care common to all patients. Prolonged CCU care was given only to patients with certain complications, thus resulting in a selection.

Policy of treatment. The treatment of AF was primarily guided by the ventricular rates. Patients with rapid ventricular rates, i.e. over 120/min, were treated with digitalis (lanatoside-C 0.4–0.8 mg or ouabain 0.25–0.38 mg i.v.) which, if not successful in reducing the ventricular rates within 1–2 hours, was supplemented by direct current electroconversion (100–300 joules) under light general anaesthesia. In severe haemodynamic dysfunction DC conversion was immediately performed, occasionally under diazepam sedation. AF of long-standing or of recurrent type was not treated with DC conversion. DC conversion was immediately performed in the presence of cerebral symptoms, hypotension, heart failure or anginal pain.

AF with ventricular rates lower than 50/min and cases with cerebral symptoms in association with ventricular rates lower than 80/min were treated with digitalis withdrawal and atropine sulphate or methyl scopolamine.

MATERIAL

During the period studied, i.e. Jan. 1 1968 until Dec. 31 1969 there were 1 099 admissions to the CCU and in 450 (41%) a diagnosis of AMI was made according to previously given criteria (8).

In the infarction group of 450 admissions there were 284 men (63%), mean age 63 years, and 166 (37%) women, mean age 71 years. Age and sex distribution as well as the mortality are shown in Fig. 1. Forty-seven patients (10%) died in the CCU and a further 48 (11%) during after-care, giving a total hospital mortality of 21%. The mean CCU stay lasted for 53 hours and the duration of hospitalization 21 days. Forty per cent of the patients were admitted within 3 hours of onset of symptoms, 62% within 6 hours and 74% within 12 hours.

RESULTS

During the first 24 hours in the CCU AF was observed in 69 (15%) of the 450 patients. It was

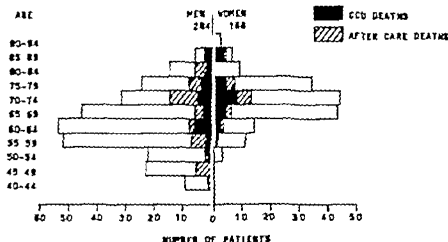


Fig. 1 Relationship between mortality and age in 450 patients with AMI treated in the CCU and subsequently in general hospital wards or a special after-care unit.

present on admission in 48 (11%) and developed during the first 4 hours in another 21 (5%).

There were 34 men aged 40–89 years (mean 69 years) and 35 women aged 57–92 years (mean 73 years). The mean age of the whole group was 71 years. The higher incidence of AF in the older age groups is shown in Fig. 2, both for the 21 patients developing AF in the CCU and for

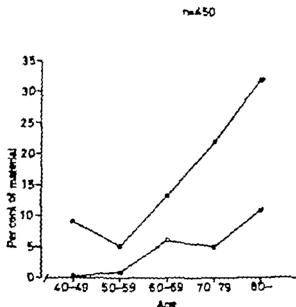


Fig. 2 AF in relation to age in 69 patients with AMI (n=450) during the first 4 hours in the CCU ● All patients with AF ○ patients with onset of AF after admission.

all 69 patients. There was a significant over representation of women ($p<0.001$).

Thirteen (19%) of the 69 patients with AF died during their CCU stay compared to 34 (9%) of the remaining 381 patients ($p<0.05$). The hospital mortality for the patients with AF (26/69 i.e. 38%) similarly exceeded that of the remainder (69/381 i.e. 18%) significantly ($p<0.001$).

Further analysis reveals that the mortality of the patients with AF aged 69 years or less, or 33% (9/27), is significantly higher than that, 12% (30/250), of the patients of the same age group but without AF ($p<0.01$). In contrast no *significantly raised mortality for the older patients* with AF (40% 17/42) was seen when compared with those without AF (30% 39/131).

Eighteen (26%) of the AF patients gave a history of at least one previous hospital-treated myocardial infarction, an incidence of the same order as amongst the remaining patients (33%).

The time elapsing between onset of symptoms leading to admission and arrival at hospital was uncertain in 6 patients. Of those with known delay 49 (71%) had arrived within the first 6 hours, which significantly differs from the figure of 61% for the remainder of the patients ($p<0.05$).

Enzymes. The results of maximum enzyme levels and the mortality rate are given in Table I. The distribution is similar in patients with and

Table I. *SGOT maximum levels and mortality for patients with and without AF during the first 24 hours in the CCU*

SGOT max. (U)	With AF				Without AF				Mortality significance
	Admissions		Deaths		Admissions		Deaths		
	No.	%	No.	%	No.	%	No.	%	
1–99	25	36	4	16	145	38	13	9	N.S.
100–299	29	42	9	31	171	45	13	8	$p<0.001$
> 300	10	14	8	80	37	10	15	41	N.S.
Not obtained[a]	5	7	5	100	28	7	28	100	N.S.
Total	69	100	26	38	381	100	69	18	$p<0.001$

Died prior to calculated enzyme maximum.

without AF. The mortality rate for the patients with AF was generally on a higher level and reached significance as compared to those without AF for patients with SGOT maximum 100–299 U ($p<0.001$). There was no overrepresentation of AF among larger infarctions.

ECG site of infarction. The ECG findings are presented in Table II and show that AF was significantly more common only in patients with uncertain ECG findings. Therefore an analysis was performed of the age distribution of uncertain ECG findings, showing that of 188 patients aged below 65 years 22% had these inconclusive ECG findings as compared to 53% among 262 patients aged 65 years and above ($p<0.001$).

Left heart failure. Findings of left heart failure during the first 24 hours in the CCU in relation to the occurrence of AF are given in Table III. Heart failure was slightly more common ($p<0.05$) among patients with AF (80%) than in the remaining 381 patients (67%). In the patients without heart failure AF did not significantly affect mortality. On the other hand AF in the presence of heart failure carried a higher mortality ($p<0.01$) (42%) when compared to the patients with heart failure but without AF (23%).

Hypotension and shock. Eleven (16%) of the patients with AF were hypotensive but not in shock, which does not significantly differ from the incidence of 11% amongst the remaining patients. Nor was any difference found when comparing the mortality in the patients with AF and hypotension (45%) with that for patients with hypotension only (34%). There were 38 patients with shock in the total group of 450. In the group with AF eight (12%) had shock, all of whom died. The patients with AF did not differ significantly from the remainder in respect either of incidence of shock or of related mortality.

Cardiac murmurs. A systolic murmur suggesting mitral regurgitation was heard in 12/69 (17%) patients with AF which differs significantly from the 6% in the remainder ($p<0.01$).

Temporal pattern. The temporal pattern of the arrhythmia is presented in Table IV which also gives rates of reversal to sinus rhythm (SR). It is seen that 67% of the 69 patients were in SR prior to discharge from the CCU or death. Repetitive episodes of AF were not associated with a raised mortality (8%). In 11 of the 69 patients, periods of atrial flutter were also observed. The mortality of the patients with AF on admission (42%) did not significantly differ from that of the patients who developed AF after admission (29%).

Table II. *Infarction site according to ECG for 69 patients with AF as compared to 381 patients without AF during the first 24 hours in the CCU*

ECG site of infarction	With AF		Without AF		Significance
	No.	%	No.	%	
Uncertain[a]	41	59	139	36	$p<0.001$
Anterior	10	14	100	26	N.S.
Anterolateral	6	9	28	7	N.S.
Inferior	6	9	57	15	N.S.
Inferolateral	4	6	33	9	N.S.
Anteroinferior	0	0	6	2	N.S.
Lateral	2	3	12	3	N.S.
Combined[b]	0	0	6	2	N.S.
Total	69	100	381	100	

Includes BBB and subendocardial infarction.
Indicates ECG changes over anterior, inferior and lateral walls.

Table III. *Heart failure and AF as seen during the first 24 hours in the CCU in relation to mortality*

	With AF				Without AF			
	Admissions		Deaths		Admissions		Deaths	
Findings	No.	%	No.	%	No.	%	No.	%
N heart failure	14	20	3	21	126	33	11	9
Heart failure (not frank pulmonary oedema)	45	65	21	47	220	58	43	20
Frank pulmonary oedema	10	14		20	35	9	15	43
Total	69	100	26		381	100	69	

Ventricular rates during AF The relationship between the ventricular rates noted and the mortality was as follows.

With persistent "low" ventricular rates (<50/min) $n=5$ mortality 20%

Normal" ventricular rates (50–119/min) $n=36$ mortality 36%

With persistent "high" ventricular rates (≥120/min) $n=28$, mortality 43%

No statistical significant differences in mortality exist when these three groups are compared with each other

Treatment The mode of therapy was regulated by the ventricular rates and the general condition of the patients (see Methods). For these reasons only a descriptive account can be given of the findings and the treatment used cannot be comparatively evaluated.

1 Patients with persistent low ventricular rates (<50 min) (*n*=*5*) Atropin or methyl scopolamine were given to all these patients. The ventricular rates rose in four One patient in shock developed persistent nodal rhythm and subsequently died. Three patients were discharged from the CCU with SR and one with AF

2. Patients with ventricular rates 50–119/min (*n*=*36*) No specific therapy was employed in 10 patients of whom 8 developed SR and 3 of these subsequently died. In two patients AF continued until death in one and until discharge from the CCU in the other Diuretics only were employed in 11 patients. SR developed in 8 and 3 subsequently died. In the other three AF continued until discharge from the CCU (1 pat.) or death (2 pats.) In addition to diuretics, 14 patients were also given a cardiac glycoside, usually because of associated severe heart failure. In 10 patients SR developed and 3 died later AF continuing in 4 with one death. Digitalis only was employed in one survivor who left the CCU with AF

3 Patients with persistent high ventricular rates (*>120/min*) (*n*=*28*) In two patients digitalis only was given. One ultimately developed SR

Table IV *Temporal pattern of AF in 69 patients with AMI during the first 24 hours in the CCU*

	With AF on admission				Without AF on admission			
Temporal pattern	No.	%	Deaths	SR before discharge from or death in CCU	No.	%	Deaths	SR before discharge from or death in CCU
One period of AF ceasing during first 24 h	25	36	8	22	9	13	3	9
One period of AF until death during first 24 h	5	7	5	0	1	1	1	1
One period of AF continuing into next day	14	20	6	1	3	4	2	3
Two or more episodes of AF during first 24 h	4	6	1	4	8	12	0	6
Total	48	70	20	27	21	30	6	19

after repeated attacks of AF whereas the other died without reverting to SR. Digitalis and diuretics were given to 18 patients. SR ensued in 8 two dying subsequently AF continued until death in 5 patients. DC conversion was resorted to in 5 patients after initial treatment with digitalis and diuretics. Two patients responded to the initial discharge (100 joules) and one subsequently died. In the other 3 the energy levels were successively raised to 300 joules with resulting SR in only one, whereas another developed SR soon after attempted conversion. The third patient died with AF DC conversion was resorted to in a further two patients without initial attempt with digitalis or diuretics. One developed SR on the first attempt (100 joules), whereas the other failed to respond to energy discharges of up to 300 joules but later reverted to SR.

No specific treatment was given to two patients with AF and high ventricular rates due to the very short duration of the arrhythmias. Both patients survived. Four patients were given diuretics only In two SR developed and they survived, whereas the other two died with AF

DISCUSSION

The incidence of AF of 15% in the present series of 450 patients admitted with AMI corresponds to the upper range of previous reports (7–16%), as recently shown in a review by Klass and Haywood (5). These reports, in contrast to the present study are generally not limited to the first 24 hours of observation. It should be added that in the present study no attempt has been made to exclude any patients who may have had AF previously

The increased mortality associated with AF differs from certain previous reports (2, 3 4 7 11) in which no increase in mortality was found. In contrast, others (5 6, 10) point to a poor prognosis in patients with AF in AMI. Still, both Stannard and Sloman (10) and Klass and Haywood (5) regard AF as a "benign arrhythmia and consider the prognosis to be related to the clinical severity of the infarction and concurrent complications.

A very clear increase in incidence of AF with rising age was found in the present study which is in accordance with the findings of Sloman et al. (9). They furthermore point to the absence of a similar relationship as regards the ventricular arrhythmias. This may result from different causal mechanisms, as has also been suggested by Lown et al. (6). The latter authors regard the atrial tachyarrhythmias as being arrhythmias of pump failure in contrast to the arrhythmias of electric instability i.e. ventricular arrhythmias. No causal relationship between AF pump failure and increasing age can be discovered from the present study Still, AF was found to herald a poor prognosis especially in the younger patients, i.e. those aged 69 years or less, whereas it did not significantly affect the prognosis in the older patients.

The most commonly associated complication of patients with AF in this series was left heart failure which was seen in 80% of the cases. Similar high values have been reported by Julian et al. (4) and Klass and Haywood (5). This relationship has often been discussed and it remains uncertain whether AF is primary secondary or both.

Another feature of patients with AF is their higher incidence of uncertain ECG findings. BBB as well as ST–T segment changes, suggestive of subendocardial infarction, are included into the group of uncertain findings in this study On the other hand uncertain ECG findings seem to be a feature of older age. Like most authors, including Stannard and Sloman (10) and Klass and Haywood (5), we ha e found no significant differences as regards the number of cases with AF and involvement of the anterior as compared to the inferior wall. Some workers (2, 3) have pointed to a rather high rate of engagement of the anterior wall in patients with supra entricular arrhythmias. Their comparatively low rate of uncertain ECG findings suggests that different criteria may have been used.

The duration of AF has not been investigated in detail in the present study Our results, on the other hand, do not contradict the general opinion that in the majority of cases the duration does not exceed 24 hours. The rate of recurrence observed during 24 hours was 17% but no comparable figures exist in the literature. Klass and Haywood (5) in their series of 31 patients found that 23% had further attacks of AF more than one day after the initial arrhythmia.

As the form of treatment employed was dictated by the ventricular rates as well as by the condi-

tion of the patient, this study does not lend itself to conclusions as to the suitability of one form of treatment as opposed to another The effect of treatment is also very difficult to evaluate as AF in AMI often ceases spontaneously A treatment under debate is DC conversion in these patients. Only 4 out of 7 in the present series responded with reversal to SR. The apparent spontaneous reversal to SR soon after attempted electroconversion in 2 patients is of interest. This low rate of success is similar to that described by Jewitt et al. (3), who consider that DC conversion is rarely indicated in this situation.

The value of mortality figures related to specific arrhythmias has been questioned especially as several workers have pointed to the fact that CCU mortality is mainly related to the severity of the infarction as judged by clinical, haemodynamic and biochemical criteria (2, 11). Accordingly two groups of authors (5 10) although finding AF to be associated with a raised mortality consider this finding as secondary to concurrent complications. Stannard and Sloman (10) thus prefer to consider the arrhythmia as "benign The present authors feel somewhat reluctant to adopt this approach. AF is admittedly not a feature of the terminal phases, in contrast to certain bradyarrhythmias and ventricular fibrillation. Nor is AF associated with severe infarctions as judged by the incidence of shock, hypotension, frank pulmonary oedema, or large infarctions as measured by maximum enzyme levels. Still, its relationship with a raised mortality remains, and it is always of potential value to find objective parameters which help us to pinpoint high-risk cases.

ACKNOWLEDGEMENT

This study was supported by grants from the Swedish National Association against Heart and Chest Diseases.

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Acta med. scand. Vol. 193 pp. 45–48 1973

DURATION OF SMOKING AND QUANTITY OF TOBACCO USED BY PATIENTS WITH GASTRIC CANCER

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Abstract. 221 patients with gastric cancer (g.c.), 174 of them current or ex-smokers, have been questioned about their smoking habits. Data regarding 157 patients (131 men and 26 women) who had smoked more than a total of 20 kg have been analysed. While the women start smoking at different ages, the men start early at approximately the same age, the majority before 16. The average duration of smoking was 48 years for men and 37 years for women. The average quantity of tobacco used was 378 kg for men and 176 kg for women. Among the women there is a marked dispersion in respect to the duration of smoking as well as the age on admission for g.c. Among the men there was an accumulation of admissions around the age of 64, i.e. after an average of 48 years of smoking. Thus in men there is a definite concurrence between the duration of smoking and the time of admission for g.c. In men over 60 years there is a statistically significant relation between the average total amount of tobacco used and the time of admission for g.c., independent of the patient's age. It is concluded that the use of tobacco may be one of the causes of the higher frequency of g.c. in men than in women.

Tobacco smoke contains several well known carcinogens (4). The unburned tobacco contains tumour-promoting substances (1) which, after being dissolved in the saliva and swallowed, may act upon the gastric mucosa. Among the many factors which may play an aetiological role in gastric cancer (g.c.) tobacco therefore seems to be one of the most probable.

If so a certain relationship might be expected between the duration of smoking, the total consumption of tobacco and the time at which g.c. arises. The present paper aims at the elucidation of this point.

MATERIAL

Among the patients with g.c. admitted to the Surgical Department of the Finsen Institute, Copenhagen, during the period 1.9.1948–31.8.1969 221 patients were questioned in detail about their smoking habits, the majority during the latter part of the period. Of these patients 174 (143 men and 31 women) were or had been smokers. Patients who had smoked less than a total of 20 kg were excluded. Data regarding the duration of smoking and the quantity of tobacco used were available for 157 patients (131 men and 26 women, presented by age and sex in Table I) who have been regular smokers and smoked more than a total of 20 kg.

The age of the men ranged from 37 to 84 years with an average of 64.2 years (±9.5). The age of the women ranged from 36 to 79 years with an average of 59.3 years (±12.1).

The amount of tobacco was calculated as follows: 1 cigarette = 1 g, 1 cheroot = 4 g, 1 cigar = 7 g, 1 pack pipe tobacco = 50 g, 1 fill = 3 g. Snuff and chewing tobacco were included in the analysis of duration, not of weight.

The calculation has to be based to some extent upon an estimate, as the consumption may vary in the individual person. In addition, part of the tobacco is not smoked, i.e. the butt of a cigarette or a cigar. On the other hand, people generally underestimate their consumption of tobacco by an average of about 20% (2).

RESULTS

While women start smoking at different ages, most of the male smokers start early at approximately the same age, the majority before 16.

The *average duration of smoking* in this study was 48 years for males and 37 years for females.

The *total amount of tobacco used* by the 157 smokers studied was 54 069 kg. The men had smoked 49 484 kg, the women 4 585 kg (9% of the total quantity).

The *average consumption of tobacco* was 378 kg for men and 176 kg for women (46% of the quantity used by the men).

In other words, we have not only fewer female smokers, but each of them smokes on the average far less than the male smokers (Fig. 1).

Table I. *Current or ex smokers by sex and age at admission*

Age (y)	Men	Women
30–39	1	2
40–49	9	3
50–59	27	9
60–69	58	6
70–79	32	6
80–89	4	—
Total	131	26

If tobacco is an aetiological factor in g.c., an increased incidence of the disease would be expected after a certain duration of smoking and a certain total consumption. In women, who start smoking at various times of life and who smoke only little, a sudden increase of the cases would not be expected to occur in a given age group

Fig. – shows a considerable dispersion in respect to the age at which g.c. occurs among the women Similarly there is a marked dispersion in respect to the duration of smoking. It cannot be ruled out that tobacco may have been a factor in some cases, but, if so, the dispersion of the age incidence corresponds to the dispersion in the duration of smoking.

Among the men, who smoke far more and most of whom start smoking at about the same age the majority before 16, there is a distinct peak around an average duration of smoking of 48 years (Fig. 3) There is a corresponding peak in admission age at about 64 years. This suggests that in men tobacco may be a contributory aetiological factor

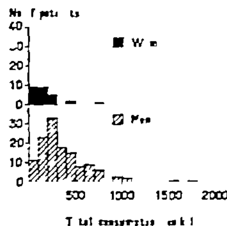


Fig. 1. Total consumption of tobacco by men and women.



Fig. 2. Relation between duration of smoking and age on admission. Women.

Fig. 4 gives a graphic presentation of the total tobacco consumption in men with g.c. in relation to their age on admission to hospital. The women, who are relatively few and who as a whole smoke little, have been left out. A considerable dispersion of the values was found.

If tobacco is of no aetiological significance, a gradually increasing total consumption would be expected with increasing age. If on the other hand, tobacco plays an aetiological role the consumption of a certain amount might be decisive and a plateau of the average values of the total consumption would be found through the age groups. As will be seen from Fig. 4 a mixture of both possibilities may be distinguished.

For men a statistical evaluation has been made of the relation between the total quantity of tobacco consumed and the age of the patients on admission. In the age group 40–60 years we find the correlation coefficient 0.29254 ($0.002 < p < 0.05$), demonstrating a correlation between age and total tobacco consumption. In the age group over 60 years we find the correlation coefficient 0.03749 ($0.05 < p$), showing no correlation between age and total tobacco consumption. The curve forms a plateau, and the inclination coefficient is 0.00195

Thus, in the age group over 60 years there is a relation between total tobacco consumption and time of admission for g.c. independent of age, thus indicating that tobacco may be of some aetiological significance in this group. In the younger age group other factors seem to be of more importance in most cases.

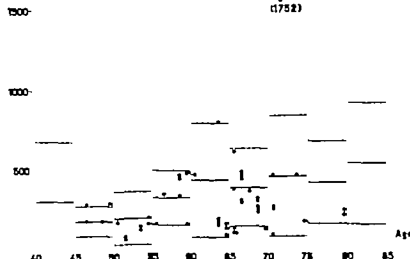


Fig 3 Relation between duration of smoking and age on admission. Men.

DISCUSSION

It has previously been demonstrated (5) that the sex ratio of smokers in the general population corresponds approximately to the sex ratio among patients with g.c. Although other factors must be operative, this arouses suspicion that tobacco plays an aetiological role.

Retrospective statistical investigations have failed to give a definite proof of the role of tobacco consumption (5), but these investigations have been obscured by the fact that many persons who later develop g.c. reduce or stop their tobacco consumption, often years before their admission to hospital for g.c. (7).

The same phenomenon has been observed in patients with pernicious anemia (p.a.), who are particularly predisposed to g.c. Many of these patients reduce or stop their tobacco consumption with increasing frequency up to the time at which treatment starts (6), probably because their ability to detoxicate the toxic substances in tobacco and tobacco smoke is impaired. Among patients with p.a., in whom g.c. has not been demonstrated, there are fewer smokers than among patients with g.c., and also fewer than among the general population (6). In patients with p.a. the sex ratio is the reverse of that for g.c., but also among patients with p.a. the incidence of g.c. is more marked among men (3). This may be explained by the higher frequency of smokers among the men.

The present investigation has demonstrated that in men there is a definite concurrence between the duration of smoking and the time of admission for g.c. Furthermore, for the age group over 60 years, a connection was found between the average amount of tobacco consumed and the time of admission for g.c., independent of the patients' ages.

These findings strengthen the suspicion of the aetiological role of tobacco, which may be one of the causes of the higher frequency of g.c. in men than in women.

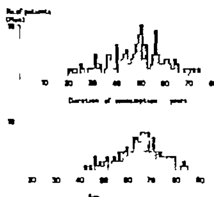


Fig 4 Relation between quantity of tobacco used and age on admission. Men.

The great dispersion of the values indicates that also among male smokers other factors must be operative as well.

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Table I. *Experimental errors*

Normal sera ($n = 20$)	Total lipid P (μg/ml)	Lecithin P (μg/ml)	Lysolecithin P (μg/ml)
Mean value	107	62	10.4
s	2.25	3.55	0.86
%	2.1	5.7	8.3

$$s = \sqrt{\frac{\Sigma(d^2)}{2n}}$$

calculated by means of linear regression analysis. A significant increase with age was found in both sexes for all the variables analyzed. The regression equations and the significance of the slope have been given in Table III. The difference between male and female values for lysolecithin and the regression lines are shown in Fig. 1.

There was no change in the relative amounts of lecithin or lysolecithin of total lipid P with age—the percentages being for lecithin 57.5 and 57.8 for men and women, respectively. For lysolecithin the corresponding figures were 10.3 and 9.2%.

DISCUSSION

Previously reported normal values for serum phospholipids generally were derived from small materials, often without the age and sex of the subjects being given. Vikrot (18) tabulated normal values from the literature and found total lipid P to vary between 57–115 mg/100 ml and lecithin and lysolecithin (as % of total phospholipids) between 61–70 and 6–11% respectively. The lowest figures for lysolecithin were found by Vikrot himself who gave several explanations, viz. that he used plasma and that the subjects were young females, together with the fact that rigorous measures were taken to avoid conversion of lecithin to lysolecithin.

Vikrot (18) found that lysolecithin levels were some 10% higher in serum than in plasma. Like others (7, 19) I found no significant difference and have thus used serum. All serum samples,

Table II. *Serum phospholipid values in various age groups*

Mean values ± S.E.M. and (below) S.D.

Age (y.) Mean	Range		ESR (mm/h)	Total lipid P (μg/ml)	Lecithin P (μg/ml)	Lecithin P (% of total lipid P)	Lysolecithin P (μg/ml)	Lysolecithin P (% of total lipid P)	Cholesterol (mg/100 ml)	Triglycerides (mmol/l)
Men										
25	23–27	10	3.4±0.8	92.5±4.8	54.2±2.3	58.6	10.1±0.58	10.9	194±7.9	0.95±0.13
			2.4	15.1	7.2		1.85		23.6	0.40
31	30–32	10	5.0±0.9	100.9±6.8	57.4±3.1	56.9	9.6±0.55	9.5	254±25.2	1.52±0.35
			2.4	21.5	9.9		1.75		79.6	1.12
40	38–43	10	4.4±0.6	101.3±3.9	60.0±2.9	59.2	11.3±0.52	11.2	252±9.3	1.45±0.24
			1.6	12.3	9.2		1.64		28.0	0.71
51	48–52	10	4.2±0.7	116.2±7.2	65.0±3.2	55.9	11.5±0.69	9.9	259±15.2	1.41±0.18
			2.0	22.9	10.3		2.17		45.7	0.53
60	57–63	10	4.8±0.7	115.9±5.2	65.9±3.1	56.9	11.8±0.51	10.2	277±21.2	1.68±0.25
			2.5	16.4	9.9		1.61		67.1	1.21
						57.5		10.3		
Women										
24	18–26	10	5.4±0.8	88.0±2.1	52.1±1.4	59.2	8.8±0.52	10.0	195±9.9	0.85±0.12
			2.5	6.7	4.5		1.64		31.2	0.38
31	29–34	10	6.1±0.8	99.9±2.8	58.3±1.8	58.4	9.1±0.56	9.1	211±8.8	0.72±0.07
			3.2	8.9	5.7		1.77		27.7	0.22
42	40–44	10	6.6±0.5	106.9±3.8	61.0±2.6	57.1	10.0±0.83	9.4	239±8.1	1.05±0.16
			2.0	11.9	8.3		2.63		25.7	0.52
51	49–52	10	5.7±0.6	120.9±3.1	68.3±2.5	56.5	10.7±0.54	8.9	275±9.3	1.14±0.13
			2.1	9.8	7.9		1.69		29.4	0.41
61	59–63	10	7.5±0.8	121.9±4.1	70.4±2.6	57.8	10.4±0.38	8.5	257±15.8	1.47±0.15
			2.6	12.9	8.2		1.20		49.9	0.46
						57.8		9.2		

Acta med. scand. Vol. 193 pp. 49–52, 1973

SERUM TOTAL PHOSPHOLIPIDS, LECITHIN AND LYSOLECITHIN —NORMAL VALUES IN VARIOUS AGE GROUPS

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Abstract. Serum phospholipids (total lipid P lecithin and lysolecithin) have been analyzed in normal men and women and found to increase to significant degree with age. Lysolecithin levels in all age groups were significantly higher in men than in women.

The analysis of individual phospholipids, e.g. lysolecithin, in serum has been considered difficult and therefore has not frequently been done (15). Most reports giving normal values are based on small materials and the results have been varying. A summary of earlier results is given in a study published by Vikrot (18). In connection with a study on the possible relationship between plasma phospholipids and the ESR (3) it was therefore found necessary to produce age- and sex-related normal values for total lipid P as well as for lecithin and lysolecithin.

MATERIAL

Serum samples were obtained from health control center (Metropol, Stockholm). All individuals underwent complete physical examination as well as routine laboratory tests (ESR, Hb, urinalysis). Only those who were found healthy and had normal Hb and ESR (Westergren method 10 mm/h) were included. All women were asked whether they were pregnant or used oral contraceptives—if the answer as yes, they were excluded from the study

METHODS

The blood samples, all obtained in the fasting state, were allowed to coagulate at room temperature during 1 hour, whereafter serum was separated, frozen and stored at −20°C until analyzed.

Cholesterol and *triglycerides* were determined according to semi-automatic method using Technicon Autoanalyzer (2, 12).

Phospholipids (total lipid P lecithin P and lysolecithin P) ere analyzed essentially according to Gottfries et al. (10). Lipids ere extracted from 0.5 ml serum by the subsequent addition of 3.5 ml methanol and 7.0 ml chloroform as described by Carlson (5). The extract was washed with 1.05 ml 0.1% KCl and allowed to stand overnight at +4°C—in glass-stoppered tubes—to ensure efficient separation. A duplicate 0.5 ml of the chloroform phase was taken for determination of total P Five ml was evaporated to dryness, the lipids were redissolved in 200 μl chloroform, and duplicate 75 μl was spotted onto freshly activated thin layer plate (silica gel H 0.25 mm). Nitrogen was used to promote evaporation during spotting.

The chromatograms were run in chloroform-methanol-water (65 25 4, volumes) in vessels protected from light and draught. The separated lipids were localized by exposure to iodine vapours. Lecithin and lysolecithin were identified by simultaneous runs of standards, purchased from Sigma Chem. Co. The spots corresponding to lecithin and lysolecithin were scraped off into tubes for phosphorous determination according to Svanborg and Svennerholm (17).

Experimental errors were calculated from duplicate analyses; the results are given in Table I.

RESULTS

The values for total serum lipid, lecithin and lysolecithin phosphorus were all apparently normally distributed. Mean values and standard error of the mean and standard deviations for the various age groups for men and women are given in Table II. Values for ESR and for serum cholesterol and triglycerides have been included in the Table.

Total lipid P and lecithin P were the same for men and women. Lysolecithin, on the other hand, was significantly lower in women than in men, absolutely as well as relatively. Lysolecithin P in men amounted, on the average, to 10.3 and in women 9.2% of total lipid P

Regression of phospholipid values on age were

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ERYTHROCYTE SEDIMENTATION RATE AND PLASMA LIPIDS

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Abstract. A highly significant positive correlation ($p < 0.001$) has been found between erythrocyte sedimentation rate (ESR) and serum cholesterol and triglycerides and—in a much smaller group—a negative correlation ($p < 0.05$) between the ESR and serum lysolecithin. These correlations exist regardless of age variations. It is suggested that it is probably the lipoprotein content of plasma that influences the ESR.

Already in 1930 Theorell (15) studied the influence of plasma lipids on the erythrocyte sedimentation rate (ESR). He found that cholesterol as well as lecithin had a strong *inhibiting* effect on the ESR and that this inhibition could be explained by an absorption of the lipids onto erythrocytes with a change in their electric charge. No correlation was found between total plasma cholesterol and the ESR, but a strong negative correlation was demonstrated between the ESR and the cholesterol that was "easily" extracted from plasma with diethylether ($r = 0.41$). Theorell found that plasma cholesterol was comparatively strongly bound to fibrinogen and "euglobulin" and advanced the theory that only "free" cholesterol would influence the aggregation of erythrocytes and thus the ESR. Further studies of the problem were carried out by Westergren et al. (16) and Ohlson and Rundqvist (13). The latter authors extracted lipids from plasma and concluded that plasma lipids exerted no or only a very small influence on the ESR. They found, however, that the increase in suspension stability of the erythrocytes found after *heating* normal plasma to +41 C for 6–8 hours, could not be demonstrated with lipid-free plasma.

The "stabilization", i.e. the decrease of the ESR after heating of plasma, was studied already by Fåhraeus (9). Recently it has been suggested that the reaction might distinguish between an elevated ESR caused by a neoplasm and that caused by an inflammation—the former group would have a low stabilization in contrast to the latter (11). This theory has been analyzed by Böttiger and Kilborn (5) who found that a slight difference could be found between these conditions, but that it could not be used for clinical purposes. The stabilization phenomenon has been extensively studied by Berlin et al. (1), who put forward the probability of lysolecithin as the responsible factor. These findings suggested that it would be worthwhile to study further the role of lipids for the ESR. This seemed even more important when it was found in a prospective study that, among patients later developing myocardial infarction, the original ESR values were higher in those with high than in those with low serum cholesterol values. Subjects with serum cholesterol > 330 mg/100 ml had a higher mean ESR (13 mm/h) than those with lower cholesterol values (8 mm/h). Further, the frequency of an elevated ESR (≥10 mm/h) was 65% in the former against 30% in the latter group. Both these differences are significant at the 5% level.

The correlation between plasma lipids and the ESR has consequently been studied in two groups of healthy people.

MATERIAL

I. The larger material—collected in 1961–62 as the basis for a prospective study—was obtained from a health survey centre (Förtagens Hälsokontroll, Stockholm). This centre makes regular examinations, at yearly intervals, of employees of various companies having a contract with the centre. Those who come for their routine visits are all engaged in active work and are subjectively healthy. Most of the individuals included in this study have been followed for several years, both before and after this particular study was started.

Table I *Composition of the material*

Group	Men	Women	Total
A	1 447	1 011	2 458
B	1 508	1 039	2 547
C	1 556	1 052	2 608
D	50	50	100

The subjects were examined physically and routine tests on blood and urine were made.

To old, as far as possible, latent atherosclerotic disease, those whose parents suffered from or died from ischaemic heart disease or cerebral vascular accidents ha e been excluded. Apart from normal physical examination and exclusion of pregnant individuals and those ith previous history of diabetes, ischaemic heart disease or thyroid or biliary disease, the following criteria were used for the selection of normals (group A).

Hb.	Men	12.8–16.8 g/100 ml
	Women	11.2 16.8 g/100 ml
BP	Systolic	170 mmHg
	Diastolic	100 mmHg

(BP readings obtained on the first visit ithout special rest.)

Urine: No sugar or protein present.

Group B = group A + those with abnormal Hb values.

Group C = group B + those ith abnormal BP values.

I all groups individuals below 20 and above 69 years of age have been excluded, the reason being that these extreme age groups contained only a few individuals (*n* = 17).

The resulting material of carefully selected normal individuals has previously been analyzed with regard to the variation of the ESR and age (7). The final composition of the groups is given in Table I.

II. A detailed analysis of serum phospholipids (total lipid P lecithin and lysolecithin) has been performed on a small group, selected in 1971 from another health survey centre (Metropol, Stockholm), working according to the same general principles as Företagens Hälsokontroll. The criteria for "normality" were the same, with the exception that no exclusions were made with regard to hereditary information, which was not vailable. It should be noted that in this group all women were asked whether they used oral contraceptives—if they did, they were excluded. This smaller material constitutes group D (Table I).

METHODS

ESR was analyzed by the Westergren method.

Serum lipids. Blood was drawn by venipuncture in the morning after fasting overnight, allowed to clot at room temperature, and the serum was separated by centrifugation.

Cholesterol was determined with the Tschugaeff reac tion in groups A, B and C and with a semi-automatic Auto-analyzer technique in group D (2).

Triglycerides were determined according to Carlson (8) in groups A, B and C and with semi-automatic Auto-analyzer technique in group D (12).

Table II *Linear regression analysis between ESR and plasma lipids and correlation and partial correlation oefficients between ESR, plasma lipids and age*

	Sex	Age (y.)		b	S_b	t	p^a	Correlation coefficients r_{xy}			Partial correlation coeff (age constant) r_{xy}	p^b
I. *ESR* (*x*) – *cholesterol* (*y*) – *age* (*z*) (*group A*)												
x y	♂	20–69	1 447	0.019	0.0021	8.76	< 0.001	0.225	0.279	0.165	0.189	< 0.01
	♀	20–69	1 011	0.021	0.0036	5.82	< 0.001	0.180	0.257	0.391	0.090	< 0.01
	♂	20–54	1 190	0.016	0.0021	7.72	< 0.001	0.218	0.248	0.183	0.182	< 0.01
	♀	20–54	895	0.017	0.0035	4.70	< 0.001	0.155	0.183	0.362	0.097	< 0.01
II. *ESR* (*x*) – *triglycerides* (*y*) – *age* (*z*) (*group B*)												
x y	♂	20–69	1 447	0.008	0.0017	5.04	< 0.001	0.131	0.279	0.022	0.131	< 0.01
	♀	20–69	1 011	0.025	0.0042	5.86	< 0.001	0.181	0.257	0.186	0.141	< 0.01
	♂	20–54	1 190	0.007	0.0016	4.40	< 0.001	0.127	0.248	0.075	0.112	< 0.01
	♀	20–54	895	0.020	0.0042	4.73	< 0.001	0.156	0.183	0.186	0.127	< 0.01
III. *ESR* (*x*) – *lysolecithin* (*y*) – *age* (*z*) (*group D*)												
x–y	♂	13–63	50	−0.084	0.175	0.48	n.s.					
	♀	18–63	50	−0.176	0.168	1.05	n.s.					
	♂+♀	18–63	100	−0.250	0.124	2.02	< 0.05	−0.200	0.177	0.329	0.277	< 0.01

[a] Statistical significance of the regression coefficient.
[b] Statistical significance of the partial correlation coefficient (age constant).

Phospholipids (total serum P lecithin and lysolecithin) were analyzed as described by Böttiger (3).

Statistical methods and symbols were used as recommended by Snedecor (14).

RESULTS

There was a highly significant, *positive* correlation ($p < 0.001$) between the ESR and serum lipid values—cholesterol and triglycerides—in men as well as in women. As both ESR and serum lipids (8) increase with age (7) partial correlation coefficients between ESR and lipids, keeping age constant, were calculated (Table II). It was found that, regardless of age, there existed a statistically significant correlation ($p < 0.01$) between the ESR values and the serum cholesterol and the serum triglyceride values, respectively

In view of the fact that plasma lipid values tend to be lower in the highest age groups (8) the correlations ha e been analyzed also for the age interval 20–54 years (Table II). It may be seen that the correlations in that group as a whole are, as might be expected from the decrease in serum lipid values, somewhat stronger than when the individuals in the upper decade are included.

Table III. *ESR and serum lysolecithin values in normal men and women (group D)*

Mean values + S.E.M. and (below) S.D.

Age (y.) Mean	Range		ESR (mm/h)	Lysolecithin (µg/ml)
Men				
25	23–27	10	3.4±0.8 2.4	10.1±0.58 1.85
31	30–32	10	5.0±0.9 2.4	9.6±0.55 1.75
40	38–43	10	4.4±0.6 1.6	11.3±0.52 1.64
51	48–52	10	4.2±0.7 2.0	11.5±0.69 2.17
60	57–63	10	4.8±0.7 2.5	11.8±0.51 1.61
Women				
24	18–26	10	5.4±0.8 2.5	8.8±0.52 1.64
31	29–34	10	6.1±0.8 3.2	9.1±0.56 1.77
42	40–44	10	6.6±0.5 2.0	10.0±0.83 2.63
51	49–52	10	5.7±0.6 2.1	10.7±0.54 1.69
61	59–63	10	7.5±0.8 2.6	10.4±0.38 1.20

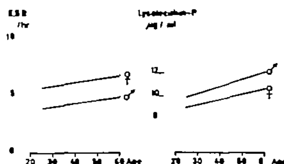


Fig. 1. Regression of ESR and serum lysolecithin values on age in normal men and omen. The corresponding regression equations are as follows:

ESR – age

♂ $y = 0.034x + 2.9$

♀ $y = 0.032x + 4.9$

Lysolecithin – age

♂ $y = 0.060x + 8.4$

♀ $y = 0.041 \ + 8.1$

The same correlations as in group A were found in groups B and C. These groups will not be further discussed.

In a previous publication (3) it has been demonstrated that serum phospholipids (total lipid P lecithin and lysolecithin) increased with age in a significant way and that serum lysolecithin values in all age groups were higher in men than in women. The complete phospholipid values were given in the previous publication, the ESR and lysolecithin values are shown in Table III.

The regression of ESR on age has been calculated in group D. The equations and the regression lines are given in Fig. 1 which also shows the corresponding values for serum lysolecithin for men and women. The ESR values in group D vary with age in exactly the same manner as in the much larger group A (7) although, due to the small material, the results do not reach statistical significance. There is, however in group D a *negative* correlation between the ESR and the serum lysolecithin values. This negative correlation is found both for men and women and reaches statistical significance ($p < 0.05$) if the values for both sexes are added together (Table II).

DISCUSSION

The results show that there is a *positive* correlation between the ESR values and serum cho-

lesterol and triglycerides, respectively and a *negative* correlation between the ESR and serum lysolecithin. The ESR rises with age as do serum cholesterol, triglyceride and phospholipid values. However the calculation of partial correlation coefficients—keeping age constant—shows that the correlation between the ESR and the serum lipid values exists regardless of age. This is therefore somewhat different from Theorell's findings as set forth in the introduction.

It should be emphasized that such a correlation does not imply a causal relationship i.e. does not state that, for example a higher cholesterol level is the cause of a higher ESR.

Variations in the composition of serum proteins have been considered the major reason for variations in ESR values, high fibrinogen and carbohydrate-rich α-2-globulin levels being especially frequently found in connection with a high ESR. Serum protein composition however does not change with age, nor do fibrinogen values. Protein influences alone could not explain the difference in ESR values between the sexes, nor the increase of the ESR with age.

A direct connection between serum lipid fractions and the ESR value has been found, inasmuch as the stabilization of the ESR through warming of plasma has been shown to be connected with an increase of plasma lysolecithin (1). This lysolecithin is formed from lecithin through enzymatic activities, probably mainly through the reaction lecithin + cholesterol − lysolecithin + cholesterol esters, a reaction catalyzed by the enzyme L-CAT (10) present in plasma.

Clinical observations, too, have made a correlation between the ESR and plasma lipid composition probable. As stated in the introduction, in subjects of a prospective study later developing myocardial infarction initial high serum cholesterol values (> 330 mg/100 ml) were found to be connected with a high frequency of elevated ESR values (4). A study in progress (6) of a large group of subjectively healthy people, in whom hyperlipaemia has been incidentally detected at a routine health control, shows a significantly higher frequency of elevated ESR values in the hyperlipaemia group than in age and sex matched controls.

The ESR is higher in women than in men—in both sexes it increases with age. The lysolecithin values increase with age in much the same manner the difference being that lysolecithin values are *lower* in women than in men. Small amounts of lysolecithin cause erythrocytes to change to a more spherical shape which decreases their ability to aggregate and thus decreases the ESR.

The sex difference in ESR may partly be explained by the difference in Hb values—even when Hb values are within the normal range there exists a close correlation between the ESR and Hb values. As discussed previously (7), the normal differences in Hb values between men and women would correspond to a difference of about 4 mm/h in the ESR value—or approximately half the normal difference. It is conceivable that the demonstrated difference in lysolecithin values could explain some part of the remaining difference.

However it would then be difficult to explain the increase of the ESR with age, as lysolecithin values increase with age—and higher lysolecithin values would mean lower ESR. A possible explanation *would be that lysolecithin in an increasing* degree—with advancing age—is bound to lipoprotein complexes and loses its effect on the erythrocyte. This is compatible with the positive correlation between ESR and cholesterol as well as triglycerides, as higher lipid values mean that more serum lipoproteins are capable of binding lysolecithin and thus abolish the ESR-decreasing effect of lysolecithin. *Such a theory regarding the effect of* "free" substances (cholesterol and phospholipids) was put forward by Theorell as early as 1930. This, in turn, would imply that it is *perhaps largely the lipoprotein content rather than* lipid or protein fractions alone that influences the ESR.

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Acta med. scand. Vol. 193, pp. 59–64 1973

COMPARISON OF THE MAIN LIPIDS IN PLATELETS AND PLASMA IN MAN

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Abstract. The concentration of total cholesterol, total phospholipids, triglycerides (TG) and free fatty acids (FFA) in plasma and platelets has been evaluated in 11 healthy male subjects. *The distribution of the different* fatty acids in FFA and TG from the two compartments was measured. All subjects had plasma lipid concentrations within the normal limits. N significant correlation could be established between the concentrations of any of the main lipid fractions in plasma and platelets. A highly significant correlation was found between FFA stearic acid in plasma and platelets, and correlation was also established for palmitoleic and oleic acids, whereas non-significant correlation was found for FFA palmitic acid. No correlation could be established for FFA polyunsaturated fatty acids or for triglyceride fatty acids.

Recent studies have established that platelets are the only formed elements of the blood capable of the novo fatty acid synthesis (8). In addition platelets are able to incorporate albumin-bound fatty acids from plasma. The incorporated fatty acids appear in platelets as free fatty acids (FFA) and in esterified form, particularly in lecithin (2, 18). At increasing concentrations of extracellular FFA considerably more of saturated than unsaturated fatty acids are taken up as platelet FFA (18). This observation is of particular relevance because it has been reported that saturated fatty acids are much more potent than unsaturated fatty acids in causing platelet aggregation (6).

Clinical studies indicate that the dietary fatty acids influence the platelet phospholipid fatty acids (14) and a high concentration of plasma triglycerides (TG) increases the platelet TG (4). However no significant correlation has been established between plasma and platelet total cholesterol and phospholipid levels (11 24), and there are marked differences between plasma and platelet phospholipid distribution (11 15).

The present study evaluates the quantity of the main lipid fractions in plasma and platelets from healthy men between 30 and 50 years of age. The composition of the triglyceride fatty acids (GFA) and the FFA in both compartments are compared.

METHODS

Preparation of platelets and plasma

Venous blood was collected from 11 healthy male subjects, aged 30–50 years, who had been fasting for at least 12 hours. Thirty-six ml blood was collected in 4 ml of 0.077 M EDTA solution, pH 6.4. By differential centrifugation platelet concentrate was prepared and washed twice (12). Platelet poor plasma was collected for lipid analysis, and the platelet button was resuspended in 10 ml washing fluid. The platelet suspension was finally centrifuged at g_{max} = 144 700 g for 30 min at 3°C in Spinco preparative ultracentrifuge, model L. The final platelet button was resuspended in 1 ml saline, and aliquots were taken off for platelet counting and protein estimation as described earlier (12). The rest of the platelet suspension was frozen and thawed three times.

Lipid analysis

Aliquots of plasma and platelet suspension were extracted by 15 ml 2 of 2 1 v/v chloroform/methanol. A detailed description of the lipid extraction procedure has been given recently (12). Total phospholipids were determined by Marinetti's modification (10) of Bartlett's method (1).

Total cholesterol was determined by gas chromatographic method (14) using 5 α-cholestane as internal standard and F & M Model 402 Hewlett Packard gas chromatograph equipped with 2 ft 4 mm i.d. glass column packed with 3.8% silicone Gum Rubber Methyl-GESE-30 on GAS-Chrom Q, 80/100 mesh and maintained on column temperature at 225°C.

TG and FFA were separated by thin layer chromato-

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Table I. *Plasma and platelet FFA in 11 normal male subjects*

Plasma (μmol/l)	Platelets (μg/10 q_{pl})	Platelets (μg/μg protein)	Regression coefficient	Correlation coefficient	Significance
500 ±141 (214–716)[b]	6.59 ±1.64 (4.3–9.6)	0.0035 ±0.0009	0.004	0.354	n.s.

S.D. [b] range.

graphy (17). Aliquots of the lipid extract were applied on silica gel chromatoplates of 'D-O' type, without $CaSO_4$ binder (Camag, Muttenz, Switzerland), with thickness of 0.5 mm, prepared according to Skipski et al. (17).

Reference compounds (The Hormel Inst., Austin, Minn., USA) included cholesteryl oleate, cholesterol, tripalmitin and stearic acid of known amounts dissolved in chloroform-methanol (2:1 /). Standards were always run parallel to unknown lipid samples.

The chromatograms were developed at room temperature with petroleum ether-diethylether-acetic acid (82 : 18 : 1, /) as solvent mixture.

The chromatograms were dried and then sprayed with rhodamine 6 G (0.05%) and viewed under ultra violet light. The appropriate areas, TG and FFA, of the mixture of standards and the samples were marked and scraped off into ampoules, and 2.0 ml boron trifluoride-methanol reagent 14% (w/v) (Applied Science Lab. Inc., State College, Pa., USA) was added. The ampoules were flushed with nitrogen, sealed and heated in boiling water for 30 min. The methyl esters were extracted into 4 ml petroleum spirit (Analar, BDH, Poole, England), followed 2 ml of 5 mol/l sodium hydroxide solution which added by drops under stirring at 0°C and collection of the upper phase. A second extraction with 2.0 ml petroleum spirit was carried out. Internal standard (The Hormel Inst., Austin, Minn., USA), heneicosanoate (21 : 0) was added to the ampoules before the methylation procedure in amounts varying between 0.03 and 0.15 μmol.

Recovery studies of ten samples of a standard lipid mixture (phosphatidyl choline, cholesterol, tripalmitin and stearic acid) gave the following percentage recovery: total lipid phosphorus 93.8±2.8, TG 86.4±5.4, fatty acids 92±2.2, cholesterol 104±2.8.

Platelet protein analysis

The protein estimation was carried out on aliquots of washed platelet concentrate as described earlier (12).

RESULTS

All subjects included in the study had levels of total cholesterol, total phospholipids, TG and FFA in plasma closely within the normal range in this laboratory.

Free fatty acids

No significant correlation could be established between the quantity of total FFA in plasma and

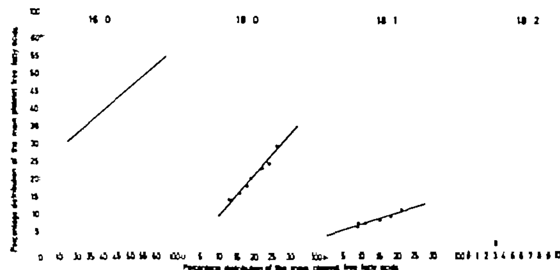


Fig. 1. Percentage distribution of palmitic, stearic, oleic and linoleic acids in plasma and platelet FFA from 11 normal men.

Table II. *Plasma and platelet TG phospholipids and total cholesterol in 11 normal male subjects*

Compound	Plasma (mg%/100 ml)	Platelets (μg/10⁹ pl)	Platelets (μg/μg protein)	Regression coefficient	Correlation coefficient	Significance
TG	73.1 ±32.0 (20–163)	9.83 ±2.63 (4.1±–15.5)	0.0040 ±0.0018	0.034	0.411	n.s.
Total phospholipids	137 ±37 (98–232)	295.0 ±52.8 (233–390)	0.16 ±0.04	−0.001	−0.024	n.s.
Total cholesterol	167 ±58 (106–304)	84.3 ±16.6 (53–105)	0.043 ±0.007	+0.141	+0.493	n.s.

platelets, with a range of plasma FFA between 214 and 716 μmol/l (Table I). However when the percentage distributions of the various fatty acids included in the FFA fractions from the two compartments were compared, a significant correlation was established for palmitoleic, stearic and oleic acids (Table III, Fig. 1). As can be seen from Fig. 1 a correlation also seemed to be present for palmitic acid, though the correlation coefficient was not significant. No correlation could be established between the polyunsaturated fatty acids.

Triglycerides

No significant correlation could be found between the total TG levels in platelets and plasma, with a range in plasma TG between 20 and 163 mg/100 ml (Table II). When the GFA were estimated, no significant correlation could be found between the two compartments, except for palmitoleic acid (Table IV Fig. 2). This may indicate that platelet TG are not directly transferred from the plasma TG but are synthesized within the platelets.

Total cholesterol

As cholesterol esters only constitute a minor part of the total platelet cholesterol pool (9), only total cholesterol was estimated. Within a plasma total cholesterol concentration of 106–304 mg/100 ml no correlation was found between platelet and plasma total cholesterol levels (Table II).

Table III. *Percentage distribution of the main fatty acids in plasma and platelet FFA*

Compound	Plasma (%)	Platelets (%)	Regression coefficient	Correlation coefficient	Significance
12 0	<1	<1			
14 0	4.8 ± 3.4	3.9 ± 2.3			
16 0	44.2 ± 9.4	43.2 ±10.0	+0.41	+0.380	n.s.
16 1	4.6 ± 2.7	4.2 ± 2.4	+0.94	+0.924	$p<0.05$
18 0	19.8 ± 3.8	20.8 ± 4.9	+1.15	+0.947	$p<0.05$
18 1	12.4 ± 5.5	8.4 ± 2.5	+0.31	+0.680	$p<0.05$
18 2	4.1 ± 2.5	2.1 ± 1.7	+0.24	+0.367	n.s.
20 0	2.1 ± 2.0	2.4 ± 1.5			
18 3	<1	<1			
<20 4	8.3 ± 4.8	11.0 ± 6.1			

Table IV *Percentage distribution of the main fatty acids in plasma and platelet TG*

Compound	Plasma ( )	Platelets (.)	Regression coefficient	Correlation coefficient	Significance
12 0	<1	<1			
14 0	2.77 ± 1 10	4.7 ± 2.3			
16:0	29.3 ± 3.9	29 7 ± 4.8	−0.11	−0.113	n.s.
16 1	6.9 ± 1 6	6.9 ± 1.3	+0.68	+0.724	$p<0.05$
18 0	7.2 ± 2.7	16.3 ± 6.3	+0.37	+0.580	n.s.
18 1	38.7 ± 2.5	.3.5 ± 3.0	−0 64	−0.512	n.s.
18:2	11.2 ± 2.6	6.6 ± 2.6	+0.44	+0.424	n.s.
20 0	<1	3.0 ± 2.2			
18 3	1.8 ± 0.8	2.6 ± 1.9			
.20 4	.7 ± .4	7.8 ± 4.9			

Total phospholipids

Earlier studies (12, 15) have shown completely different phospholipid patterns in human plasma and platelets. Within the range of 98–234 mg/100 of total plasma phospholipids no correlation could be established between the total phospholipid levels in the two compartments (Table II).

DISCUSSION

Recent studies clearly indicate that platelets are different from the other formed elements of the blood in their faculty for the novo fatty acid synthesis (8). In addition, platelets have the faculty for rapid net uptake from extracellular sources of FFA bound to albumin (2, 18). This FFA uptake seems to occur by energy independent mechanisms and, at a given extracellular FFA concentration more of the saturated fatty acids than of the unsaturated fatty acids are taken up as platelet FFA (8). Finally in vitro studies show that the extracellular FFA concentration and the FFA albumin ratio appear to be the most important factors in regulating the platelet FFA uptake.

The present study carried out in blood from healthy men with plasma FFA ranges well within the normal limits, supports the previous in vitro studies and further indicates that platelets under physiological conditions with a normal plasma FFA albumin ratio have a different affinity for each of the fatty acids. A most significant correlation was found between the FFA stearic acid content in plasma and platelets, and a correlation was also found for palmitoleic and oleic acids, whereas the correlation for palmitic acid was non-significant. It has been shown that human serum albumin has a stronger bond to oleate than to linoleate and, further that it binds palmitate more strongly than stearate (16). Thus the present observations cannot be explained only by the different affinity of albumin for the fatty acids and they support the studies of Spector et al. (18) which indicate that the platelet fatty acid affinity is the most important factor

When excessive increases in plasma TG levels were induced by i.v. fat emulsion (Intralipid) a marked increase in platelet TG occurred and intracellular deposition of lipids was observed by electron microscopy (4). The present study did not establish any correlation between the levels of plasma and platelet TG in normal individuals, and furthermore no correlation was found between the GFA concentrations. This indicates that platelet TG usually are synthesized within the platelets, whereas platelets are able to incorporate TG from plasma when excessive plasma concentrations are present. It has, however not yet been established whether patients with diseases associated with various types of hypertriglyceridemia also ha e increased concentrations of platelet TG

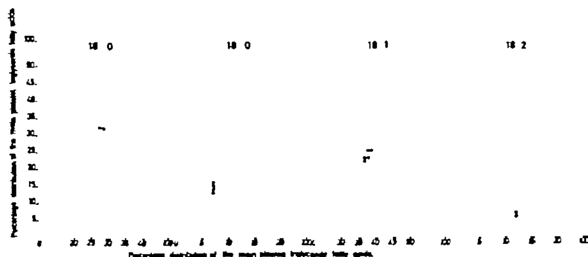


Fig. 2. Percentage distribution of palmitic, stearic, oleic and linoleic acids in plasma and platelet TG from 11 normal men.

No correlation could be established between plasma total cholesterol and phospholipid levels and the concentration of these lipid fractions in platelets. This lack of correlation has also been documented in studies with dietary-induced changes in plasma cholesterol and phospholipid concentrations, and in patients with hypercholesterolemia and hyperphospholipidemia (11 13 14). The ability of platelets to synthesize their own phospholipids is well established (6, 7), and it is suggested that platelet phospholipids and cholesterol are regulated by mechanisms independent of the plasma concentrations. It should, however be stressed that changes in dietary fatty acids are *reflected both in plasma and platelet phospholipid* fatty acids (14).

The relation between plasma lipoproteins and platelet lipoproteins is not known. There is, however good evidence that plasma lipoproteins may interfere with platelet function, most likely by interaction with the platelet membrane (3 13).

ACKNOWLEDGMENT

This work has been supported by the Norwegian Council on Cardiovascular Diseases.

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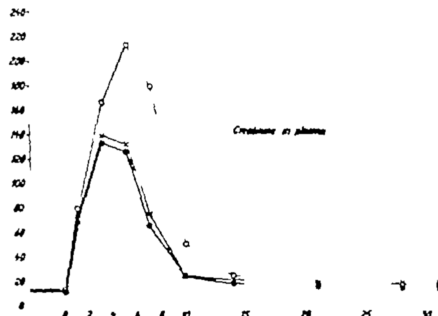


Fig. 1. Concentration of creatinine in plasma 0–31 day after transplantation in the three experimental groups. Mean values. Group A = O—O group B = ●—● group C = — The stippled line shows the values of the only surviving pig.

resistance after one hour of warm ischaemia (5), the main purpose of the present work has been to examine whether this pretreatment also improves the viability of kidneys with one hour of warm ischaemia followed by 24 hours' preservation.

Furthermore a comparison between the technically simple hypothermic storage method, as described by Collins et al. (7), and the elaborated continuous hypothermic plasma perfusion has been made with pig kidneys exposed to one hour of warm ischaemia and pretreated with chlorpromazine.

MATERIAL AND METHODS

Fifteen female pigs of the Danish Landrace breed, 4 to 5 months of age and weighing 45–68 kg at the time of surgery were used for the study. During the first 1 3 days after transplantation the animals were kept on a protein-restricted diet to which 5 g $NaHCO_3$ was added. Water was restricted until abundant urine production was observed. During the remaining experimental period the pigs were fed with a standard fodder mixture (9), and unlimited quantities of water were permitted. The animals were weighed once a week, and the daily administration of fodder was calculated on the basis of the body weight.

Preservation of the kidney

The material was divided into three groups according to pretreatment with chlorpromazine and the preservation method used, each group consisting of five pigs. In all experiments the renal artery was clamped for one hour before removal of the kidney.

Group A. About half an hour before clamping of the renal artery 500 ml 10% mannitol was given i.v. together with 1 000 ml isotonic NaCl. Five minutes before clamping, the animals were heparinized (5 000 IU heparinum NFN/10 kg b.wt.). As soon as possible after removal, the kidneys were placed in crushed glucose ice (isotonic glucose) and perfused with 200–300 ml cooled perfusate (5 C), using a 100–150 cm H_2O pressure. The perfusate was nearly similar to that described by Collins et al. (7) as the "C_4-solution", using a basic solution (containing $K_2HPO_4 3H_2O$ 9.7 g, KH_2PO_4 2.05 g, KCl 1.12 g, $NaHCO_3$ 0.84 g and aqua sterilisata ad 1 000 ml) to which papaverine (50 mg/l), glucose (20 g/l) and $MgSO_4$ (7.38 g/l) were added immediately before use. The kidneys were stored at 0°C in a solution similar to the perfusate medium, with the only exception that magnesium sulphate was omitted to prevent precipitation of heavily soluble magnesium phosphates in the storage medium.

Group B. The pigs were treated as in group A, with the only exception that 4 mg chlorpromazine/kg b.w. was added to the saline given i.v. before clamping of the renal artery.

Group C. The pigs were treated as in group B before clamping of the renal artery. As soon as possible after removal the kidneys were perfused with 200–300 ml TIS-U-SOL (containing NaCl 3.5 g, KCl 0.4 g, $MgSO_4$

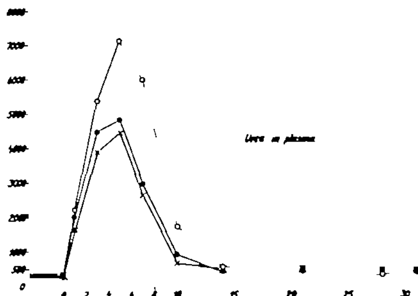


Fig. 2. Concentration of urea in plasma 0–31 days after transplantation in the three experimental groups. Mean values. Symbols as in Fig. 1.

0.2 g. $Na_2HPO_4 2H_2O$ 0.058 g. KH_2PO_4 0.0625 g. glucose 1.0 g. dextranum hydrolysatum NFN 50 g and aqua sterilisata ad 1 000 ml) with addition of 25 mg papaverine. For the next 24 hours the kidneys were preserved by continuous hypothermic plasma perfusion as described earlier (13).

After 24 hours storage the kidneys were reimplanted as described below. During surgery they were wrapped in cooled surgical dressings.

Surgical technique

The right kidney and ureter were dissected completely and the ureter cut, so that the only remaining connections between kidney and animal were the renal artery and vein. Then the renal artery was clamped and the kidney left in situ for one hour being then removed and preserved as described above. After preservation the kidney was placed at the site of the opposite and just removed kidney using end-to-end anastomosis between the renal vessels and uretero-ureteral anastomosis. For technical details see Nerstrøm et al. (16).

Peroperative studies

Thirty minutes after recirculation, renal blood flow was measured by means of the Xenon-133 wash-out technique (12). One hour after recirculation a biopsy was taken from the kidney.

Postoperative studies

Blood analyses. During the first week after the surgery blood samples were taken every second day and subsequently once a week for the following month. The concentrations in plasma of creatinine, urea, sodium and potassium were determined.

Kidney function. The clearances of inulin, endogenous creatinine, urea, para-aminohippuric acid (PAH), and the excretion percentages of water sodium, potassium and chloride were determined on unanaesthetized animals 31 days after transplantation. Each experiment comprised at least three periods of 20 min. The haematocrit values were determined. Details of the doses of test substances, the technique and the calculation methods have been published previously (9).

Analytical methods have been described previously (10).

Postmortem examination

After the observation period the animals were killed and bled, and postmortem examination was performed. The kidneys were weighed.

Histological examination

The biopsies, taken one hour after recirculation, and the necropsy specimens were fixed in neutral buffered formalin. Paraffin wax sections were stained with iron haematoxylin—van Gieson and the periodic acid Schiff reaction was carried out according to McManus and Mowry (14).

RESULTS

Groups A and B

The perfusion. After perfusion with about 300 ml perfusate it was possible to demonstrate cyanotic areas of varying size on the surface of all the kidneys. Since the animals were heparinized before arrest of the renal circulation, no further

Table I. *Renal clearances in pigs with a 4-hour preserved kidney 31 days after transplantation (group B) "Collins method"*

Pig no.	B.wt. (kg)	Relative kidney weight ( )	Haematocrit ( )	Diuresis (ml/min)	Clearance: Inulin (ml/min/10 kg b.wt.)	Clearance: Endogenous creatinine (ml/min/10 kg b.wt.)	Clearance: Urea (ml/min/10 kg b.wt.)	Clearance: PAH (ml/min/10 kg b.wt.)	Effective renal plasma flow (ml/min/10 kg b.wt.)	Effective renal blood flow (ml/min/10 kg b.wt.)
154	82	0.27	35	2.7	13	15	8	49	53	82
155	60	0.36	37	1.8	22	23	13	84	92	145
157	59	0.32	46	1.4	15	16	9	51	56	104
158	64	0.40	42	1.2	18	19	11	58	63	108
159	59	0.32	41	1.4	14	16	9	56	61	103
Average	69	0.33	40	1.7	16	18	10	60	65	109
Average of 6 pigs with no warm ischemia	68	—	38	1.9	19	1	11	72	78	127

Løkkegaard et al. (1)

attempts were made to remove blood from the vascular bed.

Initial behaviour. After recirculation most of the kidneys in both groups turned pink. Varying degrees of cyanotic areas developed in the following minutes, but after 30 min all the kidneys were nearly normal in colour and consistency. Urine production was observed within a few minutes after recirculation. Renal blood flow 30 min after recirculation was 164 ml ± 54 (S.D.) in group A and 147 ml ± .8 (S.D.) in group B. The biopsies taken one hour after recirculation revealed in both groups a microscopical picture as seen in acute tubular necrosis. The glomerular tufts showed no changes except a slight congestion and an increased number of granulocytes in most cases. In the tubules and collecting ducts many hyaline casts were found. In the proximal tubules varying degrees of cellular desquamation and vacuolated cells were seen. The interstitial tissue was slightly oedematous and inflammatory cells, especially granulocytes, were present in variable numbers. As a whole the ischaemic changes were more pronounced in group A.

Subsequent function. In group A all pigs produced some urine following autotransplantation. The mean values for the concentration of creatinine in plasma on the 3rd and 4th day after

Table II. *Renal clearances in pigs with a 24-hour preserved kidney 31 days after transplantation (Group C), continuous plasma perfusion*

Pig no.	B.wt. (kg)	Relative kidney weight (%)	Haematocrit (%)	Diuresis (ml/min)	Clearance: Inulin (ml/min/10 kg b.wt.)	Clearance: Endogenous creatinine (ml/min/10 kg b.wt.)	Clearance: Urea (ml/min/10 kg b.wt.)	Clearance: PAH (ml/min/10 kg b.wt.)	Effective renal plasma flow (ml/min/10 kg b.wt.)	Effective renal blood flow (ml/min/10 kg b.wt.)
168	72	0.35	39	2.0	13	14	9	52	57	94
169	66	0.36	40	1.4	12	14	7	44	48	80
170	64	0.37	38	1.2	17	17	8	55	60	97
176	49	0.39	38	0.5	12	13	3	32	35	56
Average	63	0.37	39	1.3	14	14	7	46	50	81
Average of 5 pigs with no warm ischemia	86	—	40	1.8	19	21	11	64	74	123

Løkkegaard et al. (13).

Clearance ratios

Cr/ In	Urea/ In	Filtration fraction In/PAH	Excretion (%) Water	Sodium	Potassium	Chloride
1.2	0.6	0.26	2.6	0.20	33.6	0.88
1.0	0.6	0.26	1.0	0.14	21.1	0.59
1.0	0.6	0.30	1.6	0.03	21.9	0.67
1.1	0.6	0.31	1.0	0.03	30.4	0.56
1.2	0.7	0.24	1.8	0.04	26.9	0.97
1.1	0.6	0.27	1.6	0.09	26.7	0.73
1.1	0.6	0.26	1.5	0.34	34.2	1.19

transplantation were 165 μg/ml ± 17 (S.D.) and 197 μg/ml ± 11 (S.D.) respectively. Four of five pigs died 4–7 days after transplantation in progressive severe renal failure (Figs. 1 and 2). Only one (no. 166) survived during the whole experimental period of 1 month. The inulin, creatinine, urea and PAH clearances for pig 166 on the 31st day after transplantation were 18, 19, 12 and 64 ml/min/10 kg, respectively.

In group B all five animals survived throughout the experimental period of 1 month. Figs. 1 and 2 show the mean concentration of creatinine and urea in plasma (μg/ml) of these five animals 0–30 days after transplantation. Mean values for the concentration of creatinine on the 3rd and 4th day after transplantation were 132 μg/ml ±

Clearance ratios

Cr/ In	Urea/ In	Filtration fraction In/PAH	Excretion (%) Water	Sodium	Potassium	Chloride
1.1	0.7	0.25	2.1	0.17	36.3	0.95
1.1	0.6	0.28	1.7	0.02	27.6	0.68
1.0	0.5	0.30	1.1	0.09	24.4	0.69
1.1	0.3	0.38	0.8	0.30	7.2	0.37
1.1	0.5	0.30	1.5	0.15	23.9	0.67
1.1	0.6	0.28	1.1	0.12	26.1	0.71

24 (S.D.) and 143 μg/ml ± 21 (S.D.), respectively. Table I shows the inulin, creatinine, urea and PAH clearances as well as the effective renal plasma and blood flow and excretion percentage for electrolytes 31 days after transplantation. Since the animals were killed immediately after the clearance experiment, it was possible to calculate the clearances both per 10 kg b.wt. and per 100 g kidney tissue (Table III). For comparison, the corresponding clearances of 4-hour identically preserved pig kidneys with practically no warm ischaemia and without pretreatment with chlorpromazine (12) are shown in Table I.

Postmortem examinations

Macroscopical findings. In group A the kidney from pig 166 was greyish-brown with many white spots on the surface. The cortex was a little swollen, with an increased content of connective tissue, the consistency was firm. In the remaining four pigs the kidneys were greyish-yellow and the cortex was markedly swollen with many yellow-white areas, especially in the deeper parts of the cortex. In all five cases the medulla had a normal appearance and the vascular and ureteral anastomoses were without complications.

In group B the kidneys had a normal colour and only a few white spots were seen on the surface. In three cases the cortex had an increased content of connective tissue, the consistency varied from normal to firm. In all five pigs the medulla had a normal appearance and the vascular and ureteral anastomoses were without complications.

Microscopical findings. In group A the kidney from pig 166 showed varying degrees of interstitial fibrosis, but was otherwise normal. The remaining four kidneys showed severe changes with focal necrosis of varying size. The proximal tubules were frequently dilated and lined with a flattened epithelium with a vacuolated appearance and pycnotic nuclei. Copious cell desquamation was seen in the proximal tubules and there were many hyaline casts in the tubules and collecting ducts. In group B all the kidneys were normal with the exception of a slight interstitial fibrosis.

Group C

The perfusion. The kidneys were perfused with a constant pulsatile perfusion pressure of about

Table III. *Average renal clearances in pigs with a kidney preserved for 24 hours after 1 hour of warm ischemia, 31 days after transplantation*

Preservation method	No. of pigs	Relative kidney weight	Haematocrit ( )	Clearance: Inulin (ml/min/10 kg b.w.t.)	Inulin (ml/min/100 g kidney)	Endogenous creatinine (ml/min/10 kg b.w.t.)	Endogenous creatinine (ml/min/100 g kidney)	Urea (ml/min/10 kg b.w.t.)	Urea (ml/min/100 g kidney)	PAH (ml/min/10 kg b.w.t.)	PAH (ml/min/100 g kidney)
*Collins (group B)	5	0.33	40	16	49 (42–62)	18	53 (48–64)	10	30 (27–36)	60	180 (144–237)
Continuous plasma perfusion (group C)	4	0.37	39	14	37 (34–45)	14	39 (33–45)	7	19 (8–25)	46	125 (80–150)

65/35 mmHg, which gave a flow rate of about 0.5–1 ml/g/min during most of the perfusion. In all cases the vascular resistance decreased somewhat during the first hours. In three cases a moderate increase was observed during the last hours of the perfusion.

Initial behaviour. The macroscopical picture just after recirculation was as described in groups A and B. Urine production started within a few minutes after recirculation. Renal blood flow 30 min after recirculation was 170 ± 33 (S.D.). The microscopical picture one hour after recirculation showed in two biopsies the same ischaemic changes as found in group B.

Subsequent function. Four animals survived the experimental period of 1 month. One died 22 days after transplantation due to pneumonia. The concentration of creatinine in plasma was 19 μg/ml at the time of death. Figs. 1 and 2 show the mean concentrations of creatinine and urea in plasma, respectively. Mean values of plasma creatinine on the 3rd and 4th day after transplantation were 139 μg/ml ± 8 (S.D.) and 156 μg/ml ± 34 (S.D.), respectively. Table II shows the inulin, creatinine, urea and PAH clearances as well as the effective renal plasma and blood flow and excretion percentage for electrolytes 31 days after transplantation. Since the animals were killed immediately after the clearance experiment it was possible to calculate the clearances both per 10 kg b.wt. and per 100 g kidney tissue (Table III). For comparison the corresponding clearances of 24-hour identically preserved pig kidneys with practically no warm ischaemia and without pretreatment with chlorpromazine (13) are shown in Table II.

Postmortem examinations

Macroscopical picture. The kidneys from pigs 168 and 175 had a normal appearance, but the consistency was a little firm. In contrast the kidneys from the three other pigs were greyish brown with varying numbers of white spots on the surface. The cortex was a little swollen and had an increased content of connective tissue; the three kidneys were firm in consistency. In all cases the medulla had a normal appearance and the vascular and ureteral anastomoses were without complications.

Microscopical picture. The kidney from pig 168 had a normal appearance. All other kidneys revealed varying degrees of interstitial fibrosis. The kidney from pig 175 (died of pneumonia) showed swollen epithelium and cell desquamation in the proximal tubules, dilated tubules and congestion of the medulla. As a whole more interstitial fibrosis was found in group C as compared to group B.

DISCUSSION

Belzer et al. (4) found a high frequency of post-transplantation renal failure in the clinic using the continuous hypothermic plasma perfusion as preservation method, and noticed that these failures often were related to a high vascular resistance of the kidneys during the perfusion. Experiments with hypothermic plasma perfusion

Effective renal blood flow	
(ml/min/ 10 kg b.wt.)	ml/min/ 100 g kidney)
100	326 (270–408)
81	223 (140–267)

of pig kidneys showed that the vascular spasm, which developed in the agonal phase, could be partly prevented if the animals were pretreated with phenoxybenzamine (4). Introducing this treatment of the donors in the clinic, the results improved essentially (3).

In experiments with rabbit kidney perfusions it has been demonstrated that the vascular resistance is 3–4 times increased after one hour of warm ischaemia, also without preceding agonal phase (11), and that this increase can be reduced by about 50% after pretreatment with chlorpromazine (5).

The results of the present study where the only difference between groups A and B was that the animals in group B were pretreated with chlorpromazine, illustrate that this treatment has a protective influence on the function of the kidneys. This was demonstrated partly by a smaller increase in the plasma concentration of creatinine in group B as compared to group A, with significant differences of the means on the 3rd ($0.025 < p < 0.05$) and on the 4th day ($0.005 < p < 0.01$) after transplantation, partly by the fact that all the animals in group B survived an experimental period of 1 month, while four of the animals in group A died in progressive renal failure 4–7 days after transplantation.

A comparison between the results in group B and earlier experiments with the same storage technique, but with practically no warm ischaemia of the kidneys and without pretreatment with chlorpromazine, shows that the depression of renal function in the first days after transplantation was of the same order of magnitude, judged from the concentration of creatinine in plasma (12). Likewise, as shown in Table I, a comparison of inulin, creatinine, urea and PAH clearances 30 days after transplantation showed no significant differences ($0.05 < p < 0.20$).

It can be concluded from these results that pretreatment with vasodilators such as phenoxybenzamine and chlorpromazine decreases the vascular resistance in kidneys during subsequent hypothermic perfusion and increases the viability of kidneys which have been exposed to ischaemia.

The measurement of renal blood flow by the Xenon-133 wash-out technique 30 min after recirculation showed no significant difference between groups A and B ($0.5 < p < 0.6$). This finding is, however, not surprising since all pigs suffered from acute tubular necrosis and since it is well known from studies on humans that measurement of renal blood flow in an early phase of tubular necrosis is of little, if any, predictive value for the severity of the lesion (15).

After pretreatment of the animals with chlorpromazine followed by one hour of warm ischaemia of the kidneys we could not demonstrate any differences between the depression in renal function in groups B and C during the first days after transplantation (Figs. 1 and 2). As shown in Table III the inulin, creatinine, urea and PAH clearances 31 days after transplantation showed higher values in group B expressed as ml/100 g kidney weight ($0.02 < p < 0.05$).

Following 15 min of warm ischaemia in dog kidneys Scott et al. (18) found the continuous hypothermic plasma perfusion to be superior to the hypothermic storage method as described by Collins et al. (7). Under the present conditions we have found no clearcut differences between the two methods in 24-hour preservation experiments.

ACKNOWLEDGEMENT

This study was supported by grant from the Ilma Foundation.

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BLOOD COAGULATION AND FIBRINOLYSIS IN RELATION TO DEGREE OF PHYSICAL ACTIVITY DURING WORK AND LEISURE TIME

A Study Based on a Random Sample of 54-year-old Men

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Abstract. In a population sample of 722 men, aged 54 years, blood coagulation and fibrinolysis have been studied in the afternoon, and later in a subsample of 76 men in the morning. Recalcification times of citrated plasma and PTT in silicone tubes were shorter and the fibrinolytic activity higher when examined in the afternoon than in the morning. The samples were subdivided into groups according to degree of physical activity during work and leisure time. Men with a high degree of physical activity during work had shorter clotting times in the afternoon and tended to have shorter clotting times also in the morning. There was no difference in fibrinolytic activity between the groups. The conclusions are that there exists a diurnal rhythm of fibrinolysis which is not linked to physical activity and that physically active men have shorter clotting times of plasma than less active men. No differences in other variables of blood clotting and fibrinolysis were found between morning and afternoon samples or between the groups with different physical activity.

Violent exercise is known to induce increased fibrinolytic activity, increased factor VIII activity and a shortened clotting time (3, 4, 7, 11, 13). Moderately increased fibrinolytic activity and increased factor VIII activity were also seen after adrenalin injection (4, 9). Fearnley et al. (5) showed a diurnal fibrinolytic rhythm, diminished activity by night and increased activity by day irrespective of whether the subjects were asleep or at work, which however could not be verified by Mathur et al. (10). The latter study indicated that the diurnal variations in fibrinolytic activity were dependent on physical activity. Some prospective population studies have shown that physical inactivity is a risk factor for myocardial infarction (16). An explanation for this may be that fibrinolytic activity is increased by physical work and that this may prevent thrombosis.

In the present study a random population sample of 788 men of the same age examined in the afternoon and a subsample of this population ($n = 76$) examined in the morning have been subdivided into groups according to degree of physical activity. Thus it has been possible to throw some light on the diurnal variations and possible effects of short-term and long-term physical activity—on blood clotting and fibrinolysis.

STUDY POPULATIONS AND METHODS

A randomly selected population sample of men born in the same year was examined. The sample consisted of all men born in 1913 on dates evenly divisible by three and living in Göteborg at the end of 196. hen the sampling took place. Thus men born on dates 3, 6, 9, 12, etc. were included. The sample thus obtained consisted of 973 men, and 855 of them (88%) ere examined at hospital in 1963 (14). Characteristics of the non-participation group have been published elsewhere (15). In 1967 803 men were reexamined. The non-respondent group consisted of 52 men, of whom 25 refused examination, 18 had died and 9 could not be traced. Because of sampling difficulties, etc. data on blood coagulation and fibrinolysis ere only available for 772 men. The blood sampling was made after hour rest at about 3 p.m. and the participants had been fasting since 12 noon on the same day.

From this sample a subsample was obtained consisting of men born on the sixth of each month of the year. Of the 85 men belonging to this subsample 76 (89%) took part in reexamination in 1968. hen the samples ere drawn after one hour's rest at 8 a.m. The participants

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Table III. *Recalcification time of citrated plasma in relation to degree of physical activity in the total sample* ($n = 772$) (mean ± S.D.)

Physical activity during occupation	Physical activity during leisure time 1	2	3+4	Total
1	441±127 =33	413±106 =95	441±104 =35	425±110 n=163
2	425±113 =52	400±103 =206	400±89 =94	404±101 =352
3+4	407±93 =54	391±140 =140	388±85 =63	394±119 =257
Total	422±109 =139	400±116 =441	404±92 =192	405±109 =772

Table IV *PTT in silicone tubes in relation to degree of physical activity in the total sample* ($n = 772$) (*mean* ± *S.D*)

Physical activity during occupation	Physical activity during leisure time 1	2	3+4	Total
1	235±43	218±53 =95	216±51 =35	221±51 =163
2	212±45 =52	210±46 =206	208±44 =94	210±45 =352
3+4	202±52 =54	206±45 =140	207±41 n=63	205±45 =257
Total	214±49 =139	210±50 =441	209±41 =192	211±46 =772

the study of differences between means of several groups. A value of $p \leq 0.05$ was considered as statistically significant.

occupational activity (Table VII). A similar trend, but not significant, was found for the results from the 1968 examination.

RESULTS

Mean values for the total population sample and the subsample when examined in 1967 (afternoon) and in 1968 (morning) are shown in Table II. The values for the subsample did not differ significantly from those of the larger sample at the 1967 examination. Clotting times were, however longer and fibrinolytic activity lower at the morning examination in 1968.

Recalcification in silicone tubes was statistically different for different grades of occupational but not of leisure time activity for the total sample examined in 1967 (Table III). Thus there were significant differences between occupational grades 1 and 2 and 1 and 3.

The PTT in silicone tubes showed exactly the same pattern for the activity grades—occupational grade 1 differed from grades 2 and 3 respectively (Table IV).

For fibrinolytic activity (Table V) no differences were found with respect to activity during occupation or leisure time.

The same pattern for recalcification time was found in the 1967 and 1968 subsamples as in the total sample, but the differences were not statistically significant, probably because of the small sample sizes (Table VI)

Significant differences for PTT in silicone tubes were found in the subsample at the 1967 examination between grades 1 and 3 as well as 2 and 3

DISCUSSION

Differences between the values from the examinations performed in the mornings and in the afternoons were found both with respect to clotting time and fibrinolysis. As laboratory faults can be excluded with great certainty these results indicate a diurnal rhythm for the variables in question. This might be due to differences in physical activity As there was no difference in fibrinolytic activity between acti e and less active men our findings seem to support the early finding of Fearnley et al. (5) that there is a diurnal rhythm of fibrinolysis which is not linked to physical activity

Table V *Fibrinolytic activity in relation to degree of physical activity in the total sample* ($n = 772$) (*mean* ± *S.D*)

Physical activity during occupation	Physical activity during leisure time 1	2	3+4	Total
1	136±22 =33	131±25 =95	138±31 =35	134±26 =163
2	130±22 =52	134±22 206	132±21 =94	133±22 =352
3+4	129±18 54	131±23 140	134±20 63	131±21 =257
Total	131±21 =139	132±23 441	134±23 192	133±23 =772

Table VI. *Recalcification time of citrated plasma in silicone tubes in the subsample (n = 76) in relation to degree of physical activity (mean ± S.D)*

Physical activity during occupation	Physical activity during leisure time 1	2	3 4	Total
Afternoon 1967				
1	331 ± 29 = 5	435 ± 75 = 3	477 ± 92 = 7	420 ± 96 = 15
2	355 ± 82 = 6	414 ± 99 n = 28	432 ± 123 n = 8	409 ± 102 = 43
3 + 4	441 ± 82 n = 4	346 ± 31 = 7	383 ± 75 n = 7	382 ± 74 n = 18
Total	370 _ 79 = 15	403 ± 93 = 38	431 ± 102 = 22	405 ± 95 76
Morning 1968				
1	432 ± 45 = 5	511 ± 110 = 3	484 ± 195 = 7	473 ± 141 = 15
2	443 ± 71 = 6	468 ± 84 = 29	452 ± 99 = 8	462 ± 84 = 43
3 + 4	429 ± 76 = 4	413 ± 153 = 7	399 ± 63 = 7	411 ± 104 = 18
Total	436 ± 60 = 15	463 ± 103 = 39	445 ± 128 n 32	452 ± 103 = 76

Table VII *PTT in silicone tubes in the subsample (n = 76) in relation to degree of physical activity (mean ± S.D)*

Physical activity during occupation	Physical activity during leisure time 1	2	3 + 4	Total
Afternoon 1967				
1	204 ± 18 = 5	222 ± 59 = 3	221 ± 65 = 7	216 ± 50 = 15
2	185 ± 27 = 6	211 ± 57 = 28	219 ± 50 = 8	209 ± 48 = 43
3 4	167 ± 20 = 4	163 ± 38 = 7	187 ± 33 = 7	173 ± 33 = 18
Total	187 ± 26 n = 15	203 ± 5. n = 38	209 ± 51 = 22	202 ± 48 = 76
Morning 1968				
1	236 ± 9 = 5	258 ± 23 = 3	.63 ± 42 = 7	253 ± 44 = 15
2	253 ± 31 = 6	228 ± 39 = 29	230 ± 49 = 8	232 ± 40 = 43
3 + 4	225 ± 57 = 4	224 ± 77 = 7	197 ± 34 = 7	214 ± 57 = 18
Total	40 ± 35 = 15	230 ± 46 = 39	230 ± 54 = 22	232 ± 46 = 76

Physically active men tend to have shorter plasma clotting times than less active men even in the morning. This indicates that the shorter otting times are not due to a short-time effect work just before blood sampling.

Thus a main conclusion of this investigation is that men who are physically active during work have shorter clotting times than less active men, but that they are not compensated by having higher fibrinolytic activity at rest (1). As mentioned in the introduction several authors have found increased fibrinolytic activity immediately after exercise, but so called poor responders have also been found (2) As the shortening of clotting times after exercise is as marked in these poor responders as in others, it has been proposed that they suffer from a dysbalance of their hemostatic system (4).

Our findings of shortened clotting times which were not compensated for by increased fibrinolytic activity in men physically active during work do not fit the generally accepted view that physical activity decreases the tendency to thrombosis.

At present we cannot offer any explanation of this. An analysis of variables which might confuse the connections found revealed that men who were physically active during work smoked in a higher proportion than others. Smokers had higher fibrinogen levels, but the fibrinolytic ac ti ity was unaffected, as were also the other variables of the hemostasis. Thus the present findings seem to be connected with physical activity per se.

There were no relationships in this population sample between physical activity and other factors which might affect blood coagulation or fibrinolysis, such as blood lipids or blood pressure.

It is interesting that Goldrick (6) found shorter stypven clotting time in natives of New Guinea than in white Australians These natives are assumed to be physically more active than average Australians. However the fibrinolytic activity was greatly increased in the natives, probably more than would compensate for the increased clotting times.

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Acta med. scand. Vol. 193 pp. 79–88, 1973

A CYTOTOXIC EFFECT ON MONKEY KIDNEY CELLS INDUCED BY SERA FROM PATIENTS WITH ACUTE RENAL FAILURE AND SUGGESTING ENDOTOXINAEMIA

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Abstract. Altogether 547 sera, obtained from 320 individuals in different reference groups and from 27 patients ith acute renal failure, have been studied for cytotoxicity on monkey kidney cells and, in 2 of the cases, also on human amniotic cells. The sera had characteristic cytotoxic effect in 1 (0.3%) of the 320 reference cases, cholecystectomized women, and in 11 (41%) of the 27 patients with acute renal failure. The clinical background of 10 of these 11 patients revealed peritonitis, intra-abdominal abscesses, paralytic ileus, prolonged shock and/or septicaemia, conditions hich were less common among the 16 patients with acute renal failure but without demonstrated serum cytotoxicity. The serum was most often cytotoxic in the early phase of renal insufficiency. The cytotoxicity disappeared with clinical improvement. The cellular changes produced by the cytotoxic sera were characterized by heavy granulation of the cytoplasm and, in the monkey kidney cell cultures, also by loss of the normal, elongated shape of the cells. These cellular changes resemble those seen in the same kind of cells incubated with sera from rabbits injected i. with endotoxin. From the clinical data and in accordance with the animal experiments it is concluded that the cytotoxic effect of the human sera may have been caused by endotoxinaemia.

Endotoxin, injected i.v. into rabbits, renders the serum cytotoxic in vitro to monkey kidney cells and human amniotic cells (10 12). As previously reported (11), serum cytotoxicity with a cellular effect similar to that described in the animal experiments may occur in man. out of a mixed series consisting of 60 patients in surgical and medical wards, the sera from 6 proved to be cytotoxic. Four of these 6 sera emanated from patients with acute renal failure i.e. lower nephron nephrosis, acute tubular necrosis.

This paper presents a further investigation of cases of acute renal failure for serum cytotoxicity in vitro. Sera from females with bacteriuria, from patients undergoing cholecystectomy and from registered blood donors served as a reference material.

MATERIAL

Patients ith acute renal failure. Forty-one serum samples were obtained from 27 patients, 9 females, aged 25–72, and 18 males, aged 26–77. The patients ere unselected in respect of the primary disease. The blood samples were generally collected in the morning or on the patients' arrival at the Renal Centre. Samples were obtained on 2–4 occasions from 2 females and 7 males. Twenty-one of the patients ere treated at the Medical Department B (Renal Clinic), University of Lund, and 6 at the Medical Department (Renal Ward), University of Umeå. Eighteen of the patients were treated with haemodialysis, 6 ith peritoneal dialysis (2 with both peritoneal and haemodialysis), and 4 did not require dialysis. One patient died before intended dialysis. In 17 cases samples ere obtained before the first treatment with haemodialysis had been started or within 2 days after the patients' arrival at the Renal Centre. Seventeen patients survived and 10 died. The diagnoses and further data are given in Table I.

The reference material consisted of 506 sera. 251 from 131 females in population study of asymptomatic bacteriuria, 159 from 93 female in-patients at the Surgical Department, Malmö General Hospital, and 96 from registered blood donors, mostly males.

In the population study (9) serum samples were obtained from participants in hom bacteriuria had been suspected. Repeated urinanalysis confirmed the diagnosis in 79 of the 131 females, aged 7–65 years. None of these 79 had renal insufficiency.

The 93 surgical cases underwent cholecystectomy because of concrements and/or chronic cholecystitis. All of the 93 patients ere operated on after having been on the aiting list. Thus, none of them were operated on in an acute stage. Only few of them had had febrile cystitis had been icteric. The serum creati

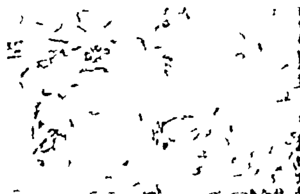


Fig 1 Cultu f monkey kidney cells in monolay photogr phed through the cultu e tube glass ithout any r ding staining procedure The cultu incubated f 4 da ith 0 1 ml human serum added to 1 ml f e medium A the cells well preserv d and kept the normal longated shap the serum classed non toxic

ol ed in at least one test in which appreciable cellular changes were observed following incubation for more than 4 days or with 0. ml serum. Eleven of these 37 sera repeatedly induced such cellular hanges, which usually appeared on the 6th 1 th day of incubation The remaining 6 sera did not reproduce the cellular changes. The indu tion of appreciable cellular changes which according to definition, were not regarded as be ing due to serum cytotoxicity was thus, in most cases, an irregular phenomenon

One (0) of the 506 sera in the reference material proved cytotoxic The cytotoxic effect was reproduced at the re test The serum was obtained on the second day after cholecystectomy from a 55-year-old woman A serum sample obtained before operation had not been cytotoxic. For 19 years the patient had occasionally had symptoms referable to the biliary tract The gall bladder proved shrivelled and a microscopic ex amination revealed chronic cholecystitis. Post operatively the serum bilirubin was mildly increased and body temperature was 38 7 C The patient did not go into shock, and her serum creatinine was normal

Thus the serum was not found to be cytotoxic among the apparently healthy individuals i e the blood donors and the non-bacteriuric females in the population study Neither did the sera prove cytotoxic in any of the cases with proved, asymptomatic bacteriuria or preoperatively in the surgical cases. However in one of the 66 chole cystectomized patients tested for cytotoxicity also postoperatively th serum was non-cytotoxic be fore but cytotoxic after the operation. The treat ment of this patient had not differed from that of the other surgical patients in the reference group.

Patients with acute renal failure

The sera from 15 (56%) of the 27 patients did not produce cytotoxic changes. Neither did re peated tests of these sera demonstrate cytotoxicity Of these 15 patients 5 were females, aged 5–72, and 10 males, aged 43–71

The serum from 1 patient (no 12) repeatedly induced cellular changes similar to those produced by cytotoxic sera but only when the culture was incubated with 0.2 ml serum. This effect was inconclusive and the serum was not regarded as cytotoxic.

Thirteen serum samples from 11 (41) of the 7 patients with acute renal failure proved cytotoxic. The results were reproduced on re testing. Figs. 1 and 2 illustrate the cytotoxic effect. Of the 11 patients exhibiting serum cytotoxicity 4 were females, aged 19–66, and 7 males, aged 6–75

Seventeen of the 7 patients recovered and 10 died within a few weeks. Serum cytotoxicity was demonstrated in 7 of the former and 4 of the latter i e. with roughly the same frequency

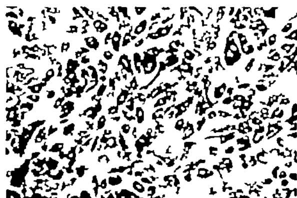


Fig Cultur of monkey kidney cell incubated fo 4 day with 0.1 ml human serum added to medium. W h out any staining procedure the cytoplasm of the cell ap pears be y granulated. The serum classed as cyto toxic A in most cytotoxic cases the granulation was pro nounced already after 4 hour of incubation. The normal elongated shape f the cells is partly lost.

Serum cytotoxicity and primary disease
The renal failure was secondary to gastro-intestinal tract disease in 9 of the 27 cases and to a disease of the biliary tract, including pancreas, in 6. The primary disease was gynaecological in 4 cases and urological in 2. Renal failure was secondary to trauma, meningococcal septicaemia, arterial embolism, drug intoxication, drug allergy and pneumonia in one case each.

Diseases within the gastro-intestinal tract. Out of the 9 patients with primary disease within the gastro-intestinal tract 8 underwent surgery. Sera from 4 of the 9 cases (nos. 1, 2, 3 and 4) proved cytotoxic. In these 4 cases intra-abdominal abscesses, peritonitis and/or paralytic ileus had preceded the development of the acute renal failure or complicated the postoperative course. The cytotoxic sera were obtained within 8 days of the development of the renal insufficiency or at the time of marked deterioration (case 3) with paralytic ileus. All 4 cases required treatment with dialysis.

Of the 5 patients with non-cytotoxic sera (nos. 13, 14, 15, 16 and 17) 2 (nos. 16 and 17) presented histories resembling those of the 4 patients with cytotoxic sera. One of these 2 (no. 16) developed a fibrinous peritonitis following a gastric resection with insufficiency of the gastro-entero-anastomosis. Acute pancreatitis complicated the course. The first serum sample from this patient was obtained on the 13th day after the gastric resection. The other patient (no. 17) had peritonitis following appendicitis. The serum sample was obtained on the 24th postoperative day. On the day before sampling the patient had had chills and fever (40.4 C), subsequent repeated culture of the blood gave growth of *Pseudomonas aeruginosa*. In this case, then, the serum proved non-cytotoxic despite septicaemia in the prolonged course of appendicitis-peritonitis.

Biliary tract diseases. Three patients (nos. 5, 6 and 18) had acute renal failure after surgery of the biliary tract. In 2 of them (nos. 5 and 6) the serum proved cytotoxic. The cytotoxicity was in both cases associated with severe infectious complications. One (no. 5) developed abscesses, subphrenic and in Douglas' pouch, after the operation. The other (no. 6) was complicated by biliary peritonitis, acute pancreatitis and septicaemia. *Proteus mirabilis* as well as coagulase-positive staphylococci were cultured from the blood.

In the 3rd patient (no. 18) the serum was not cytotoxic. The serum sample was obtained on the 22nd day after the operation; relaparotomy on the following day revealed acute pancreatitis.

Acute pancreatitis. In 3 patients (nos. 19, 20 and 21) the primary disease was acute pancreatitis. In none of them was the serum cytotoxic. Two of them died; the patient with the highest diastase value (65 000) survived. The observations in these 3 cases and in the above mentioned cases 16 and 18, in which acute pancreatitis seems to have been a main complication, thus suggest that acute pancreatitis per se is not a cause of serum cytotoxicity.

Gynaecological diseases. Four patients (nos. 7, 22, 23 and 24) had a primary gynaecological disease. One (no. 7) was subjected to total hysterectomy because of uterine carcinoma. The patient bled heavily at and after the operation and the peripheral BP could not be measured for 4 hours. A serum sample obtained on the 3rd postoperative day was cytotoxic.

No cytotoxicity of the serum was demonstrated in 3 of the 4 women. One (no. 22) had a septic abortion and shock but did not require dialysis, one (no. 23) unexplainably went into shock after an X-ray investigation (hysterosalpingography) and one (no. 24) had eclampsia of pregnancy.

Urological diseases. In 2 patients (nos. 8 and 25) the primary disease was urological. In patient 8 serum cytotoxicity was demonstrated during a paralytic ileus. Patient 25, with a hypernephroma, was complicated by severe bleeding. The serum sample, obtained on the 2nd postoperative day, was not cytotoxic.

Various primary diseases. Acute renal failure occurred in 6 patients with various diseases. The sera from 3 of them (nos. 9, 10 and 11) were cytotoxic; in 1 (no. 9) following a drug intoxication (amitriptyline) and prolonged shock without measurable peripheral BP, in 1 (no. 10) following diarrhoea and a severe generalized erythema with epidermolysis, probably as a reaction to ampicillin, and in 1 (no. 11) following a meningococcal septicaemia.

In 3 patients (nos. 12, 26 and 27) the serum was not cytotoxic. One (no. 12) had crash-landed in a glider and sustained multiple injuries. The other 2 patients (nos. 26 and 27) had acute renal failure following arterial embolism and lobar pneumonia, respectively.

Table II. *Frequency of cytotoxic sera obtained at different intervals after the patients arrival at the Renal Centre*

Day of sampling after arrival	Non-cytotoxic samples (n)	Cytotoxic samples		Total
0–1	10	6	38	16
2 3	5	4	44	9
>3	13	3	19	16
Total	28	13		41

Frequency of cytotoxicity at different intervals between admission and sampling of patients

Ten (77%) of the 13 cytotoxic sera were obtained within the first 3 days after the patients arrival at the Renal Centre. Three patients (nos. 3 5 and 9) showed serum cytotoxicity in samples collected later than on the 3rd day after arri al. Patient 3 was moribund at the time of sampling and patient 5 was in a temporarily deteriorated condition due to intra-abdominal abscesses. Patient 9 intoxicated with amitriptyline, showed serum cytotoxicity on the 6th day after arrival.

For 16 (39%) of the 41 samples the interval between admission and sampling was at most 1 day and for 25 (61%) at most 3 days, and serum cytotoxicity was demonstrated in about 40% of the samples (Table II). For 16 samples the interval was more than 3 days and the above mentioned 3 (19%) of these 16 sera were cytotoxic. Thus the frequency of serum cytotoxicity seems to decrease when the interval exceeds 3 days between admission to the Renal Centre and the sampling.

Table III. *The interval between the cytotoxic and first non-cytotoxic sample obtained in the 5 cytotoxic cases from which repeated samples were collected*

Case no.	Interval (days) Between arrival and collection of cytotoxic samples	Between arrival and collection of non-cytotoxic samples	Between collection of a cytotoxic and first non-cytotoxic sample
2	3	10	7
4	1 2 3	10	7
5	30	26 28 37	7
8	1	1 2 14	0
10	3	11	8

Repeated samples. In 9 cases samples were obtained on more than one occasion, and at least one of the samples was cytotoxic in 5 cases (nos. 2, 4 5 8 and 10) (Table III)

Case report 5

Patient 5 is selected for illustration. The cytotoxic effect was demonstrated only in the 3rd of 4 samples collected. The patient had high fever 2 days after cholecystectomy and was admitted to the Renal Centre on the 4th postoperative day. Laparotomy revealed biliary peritonitis. Twenty-six days after the cholecystectomy an abscess of the Douglas pouch was drained. The patient general condition improved gradually. The first sample to be examined for cytotoxicity was obtained 1 month after the cholecystectomy. That sample and another, obtained 2 days later were not cytotoxic. However, the patient's general condition deteriorated on the following day (3 days after the first sample), the WBC rose to 52 000/μl and emergency surgical exploration of the abdomen revealed subphrenic abscesses, which were drained. A serum sample obtained on the following day 4 days after the first sample, proved cytotoxic. After the incision the patient rapidly improved with increasing production of urine. A 4th sample, collected one week later was not cytotoxic. In this case the appearance of serum cytotoxicity fitted in with the clinical course. Culture material from the abscesses proved to be infected with *Escherichia coli* and *Pseudomonas aeruginosa*.

Repeated samples from patient 4 (Table III) showed that serum may be cytotoxic for up to 3 days. In 4 patients (nos. 2, 4 5 and 10) non-cytotoxic sera were obtained 7–8 days after cytotoxic samples. Thus serum cytotoxicity has been demonstrated to be lost within a few days of onset of acute failure, but may appear late in the course of the disease during a deterioration of the general condition.

Cytotoxicity and treatment with haemodialysis

Out of the 11 patients with demonstrated serum cytotoxicity 10 required treatment with dialysis and 1 (no. 11) died before planned dialysis. Eighteen patients were treated with haemodialysis; 9 of them showed serum cytotoxicity in samples obtained before or after the first dialysis. In 4 patients (nos. 3, 19, 20 and 23) peritoneal dialysis, but not haemodialysis, was performed; 1 of them (no. 3) showed serum cytotoxicity In 4 patients (nos. 14 22, 24 and 26) treatment with dialysis was not necessary none of them showed serum cytotoxicity

Case report 8

Patient 8 who developed acute renal failure after prostatectomy is selected for illustration because t samples ere obtained on the same day one just before the first and only treatment ith haemodialysis, and one at the end of the treatment. The first sample was cytotoxic, hile the second was not. The postoperative course was uncomplicated until the 4th postoperative day when the patient deteriorated ith paralytic ileus and excessive loss of fluid through the gastric tube. During haemodialysis 4 days later the intestines recovered their activity and the patient's general condition improved markedly. During the following days urinary excretion increased and dialysis was no longer necessary. In this patient, accordingly serum lost its cytotoxicity in association with clinical improvement during dialysis on the 8th postoperative day.

In 11 of the 18 patients treated with haemodialysis the first serum sample was obtained before the first haemodialysis, 5 of them showed cytotoxicity of that sample. In 1 of these 5 patients (no. 4) 3 samples were obtained in the early phase of the renal insufficiency period while daily dialysis was required, all 3 samples were cytotoxic.

The first sample was collected after the first haemodialysis in 7 of the 18 patients; 4 of these 7 showed serum cytotoxicity. In 3 of these 4 patients with serum cytotoxicity repeated samples were collected and found non-cytotoxic, while 2 (nos. 2 and 10) still required treatment with dialysis.

The results show that the frequency of patients with cytotoxic sera, obtained after the initial treatment with haemodialysis had been started, was of the same order as that of patients with cytotoxicity demonstrated in samples collected before the first haemodialysis.

Effect on human amniotic cells

The sera from 2 patients (nos. 4 and 7) were tested for cytotoxicity also on human amniotic cells. Both sera induced characteristic granulation in the amniotic cells, similar to that which they induced in the monkey kidney cells, and the cellular changes induced by the human sera were not found to be different from those previously induced by rabbit sera on the same kind of cells (12).

DISCUSSION

Sera from rabbits injected i. with 100–200 μg endotoxin from *Salmonella abortus equi*, *S. enteritidis*, *Escherichia coli* or *Proteus mirabilis* are cytotoxic if obtained 4–48 hours after the injection (12). The morphologically characteristic cytotoxic changes in monkey kidney cells and in human amniotic cells induced by the rabbit sera were dominated by an accentuated fatty degeneration appearing as a heavy granulation of the cytoplasm. This fatty degeneration was accompanied by modifications of form, and it appeared that the cells tended to adhere to the glass for a longer period than did control cells. The cellular changes resembled those induced by neurotoxin or so-called thermolabile endotoxin when incubated with human embryonic cells or tumour cells (22). In the previous study (12) the endotoxins prepared according to Westphal et al. (27) or Ribi et al. (23) did not make serum cytotoxic in vitro. The present study demonstrates that human serum in disease may provoke a cytotoxic effect similar to that induced by serum from endotoxin-treated rabbits. No difference was found in microscopic appearance of the cytotoxic changes produced by the human and by the rabbit sera. Neither in the animal experiments nor in the present human cases did heating to 56°C or dialysis of serum affect the cytotoxicity. Cytotoxic sera were obtained from 41% of the patients with acute renal failure.

In man endotoxins may under certain conditions, enter the blood stream from the gastrointestinal tract as well as from foci of infection in the biliary or urinary tracts (5 6, 15 21). In 1932 zu Jeddeloh (19) described cases of renal cortical necrosis following induced abortion and septicaemia. Cumulative clinical evidence has since suggested not only that bilateral renal cortical necrosis under the above circumstances is a human equivalent to the generalized Shwartzman phenomenon (17) but also that acute renal failure of the more common, reversible type without cortical necrosis, i.e. lower nephron nephrosis, acute tubular necrosis, may be due to endotoxinaemia (8 13 21 25). In the present study patients with acute renal failure were selected for investigation of serum cytotoxicity because this disease is associated with a high frequency of conditions inviting endotoxinaemia such as peritonitis, abdominal abscesses, paralytic ileus, prolonged shock and septicaemia (7).

The reference material showed that silent bacteriuria, uncomplicated gall bladder diseases such as chronic cholecystitis or concrement, as well as

Table IV. *Frequency of serum cytotoxicity related to conditions inviting endotoxinaemia in patients with acute renal failure*

A = patients with septicaemia with Gram-negative microbes, peritonitis, paralytic ileus or prolonged shock without measurable peripheral BP
B = patients without above mentioned conditions

	Time of sampling after onset of acute renal failure				
	< 8 day		> 8 day		
	A	B	A	B	Total
Cytotoxic cases	8	1	2	0	11
Non-cytotoxic cases	1	12		1	16
Total	9	13	4	1	27

abdominal surgery are not per se likely to render the serum cytotoxic. The surgical patients were included in the reference material because biliary tract diseases are common in patients with acute renal failure ( ).

The present patients with acute renal failure largely resembled those previously reported from the Renal Clinic in Lund (1, 7, 8). Endotoxin may have reached the circulation following cholecystectomy and complicating peritonitis in 2 (nos. 5 and 6) of the 11 patients with demonstrated cytotoxicity in this study. In both cases blood or material from intra-abdominal abscesses yielded growth of *Enterobacteriaceae* and or *Pseudomonas aeruginosa*. In 2 patients (nos. 1 and 2) endotoxin may have reached the circulation following peritonitis secondary to cancer of the colon and a perforated appendix. Three patients (nos. 3, 4 and 8) had paralytic ileus following gastric resection, an operation for a hernia, and prostatectomy respectively. In these cases endotoxin may have reached the circulation from the intestine. Two patients (nos. 7 and 9) had been in a state of shock for a long time without measurable BP following severe bleeding and amitriptyline intoxication, respectively. It has been well documented in animal experiments that endotoxinaemia is a consequence of prolonged shock (5, 6). One patient (no. 11) had meningococcal septicaemia, and another (no. 10) developed acute gastroenteritis and severe generalized erythema with epidermolysis after medication with ampicillin.

Paralytic ileus or peritonitis occurred in 3 (nos. 16, 17 and 23) of the 16 cases without demonstrated serum cytotoxicity (nos. 12–27). These 3 patients probably had episodes of endotoxinaemia, but the sera may have been obtained too late in the course of the disease to demonstrate any cytotoxicity. Hypotension or shock occurred in the non-cytotoxic cases 15, 16, 22, 23, 25 and 27, but a prolonged state of shock without measurable peripheral BP was not noted in the non-cytotoxic cases. The obvious difference in the clinical characteristics between patients with and without cytotoxic serum is illustrated in Table IV. Conditions inviting endotoxinaemia were found in 8 of the 9 cytotoxic cases from which samples were obtained within 8 days of the onset of acute renal failure, but in only 1 of the 13 comparable non-cytotoxic cases ($p \leq 0.002$).

The cytotoxic effect of serum may be due to vasoconstriction and impaired tissue perfusion or to release of lysosomal enzymes following endotoxinaemia (18). In rabbits a sublethal i.v. dose of radioactively labelled endotoxin disappears almost completely from the circulation within one hour (4, 16). The endotoxin is taken up mainly by the reticuloendothelial cells of the liver and spleen. Judging from animal experiments, endotoxinaemia may be only a transient phenomenon of short duration. A test for demonstrating circulating endotoxin would presumably be of limited value for deciding whether, for instance, acute renal failure may be due to an episode of endotoxinaemia in a given case.

Used as a test for a systemic effect of endotoxin the generated cytotoxicity of serum may be compared with the hyperreactivity to vasopressor substances induced by the endotoxins. The epinephrine skin test—which produces a local dermal necrosis in rabbits injected i.v. with endotoxin (24)—has been performed also in man (3, 7, 14). Of 65 patients with acute renal failure 20 (31%) reacted positively (7). In accordance with the positive epinephrine test serum cytotoxicity was most often observed within the first days of the patients' arrival at the Renal Centre. In this study the epinephrine test was used in 2 patients (nos. 2 and 8), both of whom—in accordance with serum cytotoxicity—reacted positively to intradermal injection of 0.1 mg epinephrine

given at the time of collection of the first blood sample.

Dermal necrosis following infusion of norepinephrine has earlier been described in acute renal failure and associated with conditions inviting endotoxinaemia (8). The author has previously observed similar necrosis also after i.v. infusion of metaraminol. Two of the present patients (nos. 8 and 16) developed dermal necrosis following infusion of metaraminol.

Despite existing dermal necrosis due to norepinephrine this substance has continuously been infused without the development of new necrosis during roughly unchanged deteriorated condition of the patient (8), and the epinephrine test may be negative despite the presence of Gram-negative rods in the blood (7). In the present study serum cytotoxicity during Gram-negative septicaemia was not found in a prolonged course of appendicitis-peritonitis (pat. 17). It is known from animal experiments (28) that vascular hyper-reactivity to epinephrine is only brief following injection of large doses of endotoxin, and it is known from clinical observations (26) that a specific shock-like picture may be absent despite the presence of Gram-negative bacteriaemia. These findings may reflect a state of excessive vascular fatigue and increased tolerance to endotoxin, respectively, mechanisms which have probably been active also in some of the present cases.

Further investigation of the relationship between circulating endotoxin, hyperreactivity to epinephrine and serum cytotoxicity in man is in progress.

ACKNOWLEDGEMENTS

The investigation was supported by grants from the Royal Physiographical Society at Lund and the Medical Faculty, University of Lund.

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Acta med. scand. Vol. 193 pp. 89–96, 1973

FATTY ACID AS A DETERMINANT OF MYOCARDIAL SUBSTRATE AND OXYGEN METABOLISM IN MAN AT REST AND DURING PROLONGED EXERCISE

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Abstract. The relationship of fatty acids to the metabolism of the human heart has been investigated in 41 healthy fasting men. In some subjects low plasma free fatty acid (FFA) concentrations were produced by infusing nicotinic acid. There existed negative correlations between myocardial extraction of glucose, lactate and pyruvate and that of FFA. Furthermore significant negative linear relationships between myocardial extraction of glucose and lactate and that of FFA were also present on partial correlation analyses eliminating the influence of other carbohydrate substrates. Possible explanations for these findings were discussed. Myocardial extraction of oxygen was positively related to that of FFA at rest unrelated to heart rate. The possibility and its clinical implications, that FFA may increase myocardial oxygen requirements was discussed.

Animal experiments have suggested that fatty acids might affect myocardial metabolism in at least two ways. Firstly it has been shown that increased concentrations of free fatty acids (FFA) in the perfusate of the isolated rat heart decrease its glucose (31 36) and pyruvate (13 15) uptake and utilization. In the dog, extraction of lactate by the myocardium also appears to be reduced by increased plasma FFA concentrations (20). We have recently obtained evidence in support of these findings for the human heart as well (6, 27). A common explanation of these effects of FFA may be that myocardial fatty acid oxidation is stimulated, which leads to an increase in the intracellular level of acetyl coenzyme A relative to free coenzyme A, with consequent inhibition of pyruvate dehydrogenase (16) and thus reduction of the rate of glucose metabolism along the glycolytic pathway. Conversion of rat heart pyruvate dehydrogenase from an active to an inactive form by fatty acid has been reported (43). Moreover as far as glucose is concerned, the phosphofructokinase and hexokinase reactions may be inhibited by fatty acids (31) with further reduction of glycolysis. The second way in which fatty acid might affect myocardial metabolism is to increase the oxygen need at a given level of myocardial work. The oxygen consumption of the isolated rat heart has been shown to be increased by perfusion with high concentrations of FFA (10). In dogs, myocardial oxygen consumption is increased when the plasma FFA concentration is elevated by i. heparin injection during i.v infusion of fat emulsion (30).

In man, noradrenaline increases both oxygen consumption and FFA oxidation (18 40) In the isolated perfused rat heart adrenaline increases oxygen consumption (9 10 13) and, in a situation where it does not perform work, intracellular FFA levels (11). Although increased FFA oxidation is thus accompained by increased oxygen consumption, increased glucose oxidation, produced by insulin in the perfusate of the isolated heart, is not accompanied by any change in oxygen consumption (14).

In the present study relationships between fatty acid and myocardial carbohydrate and oxygen extractions in man, in situations with low normal and high arterial FFA concentrations, have been investigated.

MATERIAL AND METHODS

Design of studies

T measure substrate and oxygen differences across the coronary circulation, referred to here as myocardial extractions, brachial artery and the coronary sinus of

Table I. *Concentrations in arterial blood and myocardial extractions of various blood and plasma substrates in the four experimental categories*

Mean ± S.E.M. and number of observations (within parentheses)

	Rest		Exercise	
	Without nicotinate	Nicotinate infusion	Without nicotinate	Nicotinate infusion
Concentrations (μmol/l)				
Plasma FFA	720 ± 40 (47)	250 ± 70 (10)	1 _40 ± 100 (15)	260 ± 30 (10)
Blood glucose	4 200 ± 90 (4)	4 360 ± 150 (10)	3 440 ± 120 (15)	3 360 ± 250 (10)
Blood lactate	670 ± 40 (47)	440 ± 40 (10)	1 350 ± 140 (15)	1 520 ± 10 (10)
Blood pyruvate	33 ± 3 (47)	74 ± 8 (10)	89 ± 4 (15)	103 ± 10 (10)
Extractions (μmol/l)				
Plasma FFA	1 0 ± 10 (47)	40 ± 10 (10)	210 ± 10 (15)	20 ± 10 (10)
Blood glucose	180 ± 20 (43)	390 ± 40 (10)	120 ± 20 (15)	330 ± 40 (10)
Blood lactate	130 ± 20 (47)	3 0 ± 30 (10)	310 ± 60 (15)	540 ± 90 (10)
Blood pyruvate	7 ± 3 (47)	37 ± 8 (10)	4 ± 5 (15)	40 ± 4 (10)

41 healthy fasting male subjects were catheterized as described elsewhere (5, 25, 26). Heparin was not administered. Instead the arterial catheter was kept patent by intermittent flushing with isotonic saline and the coronary sinus catheter by a continuous slow infusion of 0.5% citrate in isotonic saline. The beginning of the study was marked by the commencement of a constant infusion of H-labelled palmitic acid complexed to human serum albumin (26) into an antecubital vein. The first blood sampling was made 60 or 90 min later. Observations were made at rest and during prolonged exercise with and without constant infusion of sodium nicotinate to block FFA mobilization from adipose tissue and hence decrease arterial FFA concentration.

The subjects exercised in the supine position on a bicycle ergometer at a constant, predetermined work load. The work load was 50% of that which produced a heart rate of 1 0/min after 6 min of exercise (W_{170}) (25, 37, 41). The exercise lasted for 65–125 min. It was intended to last for 120 min, the duration tolerated by most healthy subjects at the load used (1). However, since the subjects were fasting and some of them were infused with nicotinate, 15 of 25 stopped earlier because of fatigue. The exercise sampling was made during the last 5 min of work in all subjects.

On 16 subjects observations were made at rest at 90 and/or 120 min; on another 25 subjects one observation was made at rest at 60 min, then supine leg exercise was begun and a second observation was made during prolonged exercise. In 10 of these 25 subjects a constant infusion of sodium nicotinate (200 mg/h in 3 and 400 mg/h in 7 subjects) was maintained throughout, after priming 1 dose of 200 mg sodium nicotinate. This meant that during some observations plasma concentrations of FFA were in the normal fasting range, during others they were high, induced by prolonged exercise, and during others they were low at rest and during exercise because of the infusion of the antilipolytic agent sodium nicotinate (Table I).

Treatment of samples

Blood samples for estimation of oxygen content were drawn into heparinized glass syringes and other samples into unheparinized plastic syringes. Samples for determination of lactate and pyruvate were deproteinized immediately with perchloric acid. The absence of chylomicra, confirmation of the fasting state, was checked by paper electrophoresis of lipoproteins (28) from a sample of blood placed in an unheparinized tube. The remaining blood was transferred to heparinized tubes and aliquots for glucose determination were immediately deproteinized with perchloric acid. The heparinized whole blood was kept for 30–60 min in iced water and then centrifuged at 4°C. The plasma so obtained was either extracted immediately for determination of FFA concentration and also FFA radioactivity or stored at −20°C for subsequent determination of plasma glycerol, usually within one week.

Analytical methods

Oxygen saturation was measured spectrophotometrically (33). Oxygen tension was measured with a polarographic electrode (Instrumentation Lab. mod. 113). The oxygen content was calculated from oxygen saturation and Hb concentration together with oxygen tension (23).

Blood glucose was assayed in duplicate on each of 10 aliquots of whole blood from each sample, using a commercially available glucose oxidase method (Kabi, Stockholm) based on that of Hjelm (21). Blood lactate and pyruvate were assayed in duplicate by the enzymatic method of Lundholm et al. (29) and Bucher et al. (3), respectively. Plasma FFA were assayed in quadruplicate according to Trout et al. (40); the heptane phase was washed with 0.05% H_2SO_4 once in the studies in which only resting observations were made and twice in those in which exercise observations were made as well. Radioactivity in the plasma FFA was determined according to the method of Boberg (2, 26). Glycerol was measured in quadruplicate on each of four extracts from each blood sample by an enzymatic fluorometric method (12).

Calculations and statistical analysis

The release of FFA into the coronary sinus was taken as the difference between FFA extraction measured radioisotopically and chemically. The radioisotopic FFA extraction was calculated by dividing the arterial-coronary sinus difference in radioactivity by the arterial specific radioactivity.

Correlation, partial correlation and linear regression analyses were made according to Snedecor (38).

RESULTS

Heart Rates

The heart rate (beats/min) at rest was 70 ± 1 (mean $\pm$ S.E.M.) without and 75 ± 3 with nicotinic acid infusion at the end of exercise it was 142 ± 4 without and 149 ± 6 with infusion.

Substrate Metabolism

Mean values in four experimental categories

The means for the four categories—rest with and without nicotinate, exercise with and without nicotinate—indicate that myocardial extraction of plasma FFA follows plasma concentration (Table I). When mean FFA extraction is low during nicotinate infusion, mean glucose, lactate and pyruvate extraction is high. The order of difference in mean extraction of these carbohydrate substrates between the situations without and with nicotinate cannot be related to the difference between their mean plasma concentrations (Table 1).

Correlation analysis for rest and exercise

Linear correlation coefficients for the relationship between the myocardial extraction of a substrate ($C_{(a-cs)}$) and its arterial concentration (Ca) are shown in Table II. For FFA, lactate and pyruvate significant positive relationships exist at rest, during prolonged exercise and for rest and exercise

Table II. *Relationship between the myocardial extraction and the arterial concentration of a given substr t (correlation coefficients ( ))*

Data from the categories ith and without nicotinate infusion are combined. C_a = plasma concentration of free fatty acid and the concentration in hole blood of glucose, lactate or pyruvate; $C_{(a-cs)}$ = difference in concentration between arterial and coronary sinus blood, i.e. myocardial extraction from plasma for FFA and from hole blood for glucose, lactate and pyruvate. Number of observations within parentheses

$C_{(a-cs)}$	C	Rest and exercise	Rest	Exercise
FFA	FFA	0.72	0.75	0.84
		(82)	(57)	(25)
Glucose	Glucose	0.27[ns]	0.37	0.09[ns]
		(78)	(53)	(25)
Lactate	Lactate	0.82	0.54	0.85
		(82)	(57)	(25)
Pyruvate	Pyruvate	0.79	0.85	0.53
		(82)	(57)	(25)

ns, $p > 0.05$ p 0.05 $p < 0.01$ $p < 0.001$

Table III. *Relationships between extractions from coronary blood of substrates for myocardial metabolism (correlation coefficients (r))*

Symbols and abbreviations as in Table II

$C_{(a-cs)}$	$C_{(a-cs)}$	Rest and exercise	Rest	Exercise
Glucose	FFA	−0.49	−0.44	−0.61
		(78)	(53)	(25)
Lactate	FFA	−0.44	−0.50	−0.43
		(82)	(57)	(25)
Pyruvate	FFA	−0.40	−0.38	−0.46
		(82)	(57)	(25)
Lactate	Glucose	0.29	0.44	0.32[ns]
		(78)	(53)	(25)
Pyruvate	Glucose	0.26	0.34	0.14[ns]
		(78)	(53)	(25)
Pyruvate	Lactate	0.62	0.69	0.47
		(82)	(57)	(25)

considered together For glucose, however a significant relationship is found only for the resting observations. A limitation of such an analysis in the case of the combined observations is that, for example, an increase in coronary blood flow could lead to an increased myocardial uptake (extraction × flow) of a substrate while blood concentration and extraction were unaltered.

However this is not a problem where the extraction of one substrate is related to that of another since the related extractions take place at the same blood flow With this approach significant negative correlations are to be found between the myocardial extractions of glucose lactate and pyruvate and that of FFA in all instances (Table III). With the exceptions of glucose/FFA overall and of pyruvate/FFA at rest, there are no significant relationships between plasma concentrations underlying the xtraction relationships (Table IV)

The significant positi e correlations, overall and at rest, between glucose extraction and the extractions of lactate and pyru ate could depend on their mutually negative correlations with FFA extraction (Tables III and V) The significant correlations to be found between the arterial concentrations of glucose and those of lactate and of pyruvate are actually negati e (Table IV) and therefore cannot underlie the positive correlations for the extractions (Table III)

The significant positi e correlations between lactat extraction and pyruvate extraction ha e

Table IV *Relationships between concentrations in arterial blood of substrates (correlation coefficients (r))*

Symbols and abbreviations as in Table II

C_a	C	Rest and exercise	Rest	Exercise
Glucose	FFA	−0.23 (78)	-0.11^{NS} (53)	-0.10^{NS} (25)
Lactate	FFA	0.03^{NS} (82)	-0.25^{NS} (57)	-0.17^{NS} (25)
Pyruvate	FFA	-0.05^{NS} (82)	−0.34 (57)	-0.22^{NS} (25)
Lactate	Glucose	−0.32 (78)	0.20^{NS} (53)	-0.03^{NS} (25)
Pyruvate	Glucose	−0.43 (78)	-0.02^{NS} (53)	−0.50 (25)
Pyruvate	Lactate	0.68 (82)	0.45 (57)	0.62 (25)

been observed by others (35) They could be accounted for in part by or explain in part, mutually negative correlations of $C_{(a-cs)}$ lactate and $C_{(a-cs)}$ pyruvate with $C_{(a-cs)}$ FFA (Table III) the positive correlations that concentrations of lactate and pyruvate bear to each other (Table IV) could also underlie the positive correlations for the extractions (but see below and Table V).

Partial correlation analysis

Partial correlation analysis shows that the myocardial extractions of FFA and glucose are related irrespective of the extractions of lactate and pyruvate (Table V) The relationship between the myocardial extractions of FFA and lactate at rest also remains significant when the effects of both glucose and pyruvate extractions are eliminated this does not apply however to the relationship during exercise, which becomes non-significant. Relationships between FFA and pyruvate extractions are not significant when both glucose and lactate extractions are held constant.

The extractions of pyruvate and lactate are closely related for the overall data and at rest when the extractions of FFA and glucose are held constant. The relationship between pyruvate and lactate extractions is not less significant when the arterial concentrations of both are held constant.

Although myocardial FFA extraction is closely related to plasma FFA concentration, it is of interest to examine the relationships between FFA extraction and concentrations of other substrates (Table VI). Apart from pyruvate concentration for which the overall data show a weak correlation, carbohydrate substrate concentrations do not bear significant relationships to FFA extraction. This implies that either plasma FFA concentration or the myocardial extraction of FFA can be predictive of the myocardial extraction of carbohydrate.

Oxygen Metabolism

FFA

For the resting and exercise observations combined there is a significant positive correlation on

Table V *Relationships between extractions from coronary blood of substrates for myocardial metabolism—partial correlation analysis (partial correlation coefficients)*

Symbols and abbreviations as in Table II

$C_{(a-cs)}$	$C_{(a-cs)}$	Eliminating $C_{(a-cs)}$	Rest and exercise (n = 78)	Rest (n = 53)	Exercise (n = 25)
Glucose	FFA	Lactate + pyruvate	−0.41	−0.28	−0.59
Lactate	FFA	Glucose + pyruvate	-0.22^{NS}	−0.29	-0.13^{NS}
Pyruvate	FFA	Glucose + lactate	-0.15^{NS}	-0.03^{NS}	-0.38^{NS}
Lactate	Glucose	FFA + pyruvate	0.06^{NS}	0.20^{NS}	0.16^{NS}
Pyruvate	Glucose	FFA + lactate	0.04^{NS}	0.05^{NS}	-0.21^{NS}
Pyruvate	Lactate	FFA + glucose	0.53	0.61	0.37^{NS}
Pyruvate	Lactate	C_a pyruvate + C_a lactate	0.47	0.52	0.43

Table VI. *Relationships between the myocardial extraction of FFA and the concentrations in arterial blood of substrates (correlation coefficients (r))*

Symbols and abbreviations as in Table II

$C_{(a-cs)}$	C_a	Rest and exercise	Rest	Exercise
FFA	Glucose	$-0.00^{n.s.}$ (78)	$-0.09^{n.s.}$ (53)	$-0.05^{n.s.}$ (25)
FFA	Lactate	$-0.15^{n.s.}$ (82)	$-0.10^{n.s.}$ (57)	$-0.14^{n.s.}$ (25)
FFA	Pyruvate	-0.24 (82)	$-0.24^{n.s.}$ (57)	$-0.21^{n.s.}$ (25)

linear regression analyses between the myocardial extractions of oxygen and FFA from coronary blood (Table VII). For the resting observations alone the relationship is highly significant (Table VII, Fig. 1) but for the exercise observations alone it is not significant. However when only those observations where no infusion of sodium nicotinate was given are considered, the relationship at rest remains highly significant and that during prolonged exercise is significant (Table VII, Fig. 1). The observations which do not appear to conform to the general relationship are, therefore, those during prolonged exercise in the presence of an infusion of nicotinate.

Carbohydrate

Myocardial oxygen extraction is not significantly related to glucose or pyruvate extraction (Table VII). However myocardial oxygen extraction is significantly and positively correlated with lactate extraction when the resting and exercise observations are considered together but not when these observations are considered separately (Table VII).

Intramyocardial lipolysis

Myocardial oxygen extraction has a relationship to glycerol extraction (or glycerol release) which appears to be exercise-dependent (Table VII). The more glycerol released, as in exercise in the absence of nicotinate infusion, the greater the oxygen extraction. Glycerol release during exercise could be a reflection of intramyocardial lipolysis. "FFA release" into the coronary circulation, taken as the difference between actual FFA uptake estimated radioisotopically and net FFA

Table VII. *Relationships between myocardial oxygen extraction and the extractions of various myocardial substrates (correlation coefficients (r))*

Symbols and abbreviations as in Table II

$C_{(a-cs)}$	$C_{(a-cs)}$	Rest and exercise	Rest	Exercise
Oxygen	FFA	0.25 (82)	0.45 (57)	$0.17^{n.s.}$ (25)
Oxygen	FFA[b]	0.57 (62)	0.53 (47)	0.55 (15)
Oxygen	Glucose	$0.04^{n.s.}$ (78)	$0.07^{n.s.}$ (53)	$0.08^{n.s.}$ (25)
Oxygen	Lactate	0.33 (82)	$0.04^{n.s.}$ (57)	$0.19^{n.s.}$ (25)
Oxygen	Pyruvate	$0.18^{n.s.}$ (82)	$0.01^{n.s.}$ (57)	$-0.01^{n.s.}$ (25)
Oxygen	Glycerol[c]	-0.26 (82)	$0.04^{n.s.}$ (57)	$-0.15^{n.s.}$ (25)
Oxygen	FFA release[d]	$-0.$[illegible] (77)	$-0.06^{n.s.}$ (52)	$-0.02^{n.s.}$ (25)

All data.
[b] All data excluding those with nicotinate.
Becomes consistently negative during exercise without nicotinate infusion, i.e. glycerol release into the coronary circulation occurs (25).
The difference between actual FFA extraction measured radioisotopically (25, 26) and net FFA extraction measured chemically.

uptake estimated chemically is inversely related to myocardial oxygen extraction, and this relationship also appears to be exercise-dependent (Table VII). The rationale for regarding intramyocardial lipolysis as a source of glycerol in the coronary sinus and of fatty acid for myocardial metabolism is presented elsewhere (25).

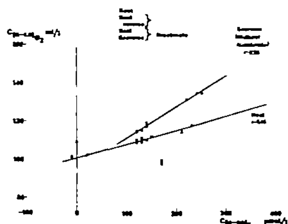


Fig. 1. Relationships between myocardial extractions of oxygen and FFA. Significance levels of regression lines drawn are shown in Table VII.

Table VIII. *Relationships between heart rate and either myocardial oxygen extraction or myocardial FFA extraction (correlation coefficients (r))*

Symbols and abbreviations as in Table II

$C_{(a-cs)}$	Rest and exercise	Rest	Exercise
Oxygen[a]	0.54	0.14^{NS}	0.40
	(82)	(57)	(25)
Oxygen[b]	0.52	0.16^{NS}	0.62
	(62)	(47)	(15)
FFA	-0.10^{NS}	-0.01^{NS}	0^{NS}
	(82)	(57)	(25)
FFA[b]	0.31	0.21^{NS}	0.52
	(62)	(47)	(15)

[a] All data. [b] All data excluding those with nicotinate.

Heart rate

At rest, myocardial oxygen extraction is not related significantly to heart rate in the present investigation, but is so for the combined resting and exercise observations and for the exercise observations alone (Table VIII). Also, at rest, heart rate is not related to $C_{(a-cs)}$ FFA (Table VIII), so that the relationship $C_{(a-cs)}$ oxygen/$C_{(a-cs)}$ FFA at rest does not arise through mutual dependence of $C_{(a-cs)}$ oxygen and $C_{(a-cs)}$ FFA on heart rate. This is confirmed by partial correlation analysis (Table IX). However, during prolonged exercise without nicotinate the relationship $C_{(a-cs)}$ oxygen $C_{(a-cs)}$ FFA does depend on changes in heart rate (Tables VIII and IX).

DISCUSSION

Substrate metabolism

From the viewpoint of cause and effect, it is of importance that sodium nicotinate has been used in these studies to induce a primary change in plasma FFA concentrations, so that differences in carbohydrate extraction between observations with and without nicotinate may be regarded as secondary to changes in FFA levels (4) although direct effects of nicotinic acid on carbohydrate metabolism has not been ruled out. The correlations, therefore, suggest that changes in myocardial FFA extraction may lead to altered extractions of glucose, lactate and pyruvate. For lactate extraction during exercise and pyruvate extraction at rest and during exercise, however, the relationships with FFA extraction (Table III) depend on interrelationships with other carbohydrate substrates (Table V).

From the present study it is not possible to say to what extent FFA regulate carbohydrate extraction by the myocardium. The low correlation coefficients suggest that other factors are involved. One possibility is that the population studied is heterogeneous with respect to the effect of FFA on carbohydrate extraction. Especially for lactate and pyruvate and, to a lesser extent, for glucose, their own blood concentrations contribute to the determination of their respective extractions. Hormones, which have not been considered here, may also modify myocardial carbohydrate extraction.

Oxygen metabolism

For the resting observations the only extraction of a myocardial substrate which is related to myocardial oxygen extraction is that of FFA. It is known that the energy obtained from a given amount of oxygen is less for lipid than for carbohydrate oxidation (42). Thus the first possibility is that FFA extraction and subsequent oxidation may in part determine myocardial oxygen requirements. This view is supported by animal studies where an increased delivery of FFA to the heart has been followed by an increased myocardial oxygen consumption (10, 30). In the present study, myocardial oxygen extraction and not oxygen consumption was measured. However, since myocardial oxygen uptake seems to be increased, like that of other muscle tissue (7, 24), by increases in blood flow (22) and oxygen extraction (25) and, since these variables seem to increase in parallel (7, 24), increased oxygen extraction probably reflects increased oxygen consumption.

At rest the myocardial oxygen extraction in the absence of nicotinate (106.5 ± 3.3 ml/l) is

Table IX. *Relationships between myocardial oxygen extraction and myocardial FFA extraction—partial correlation analysis eliminating heart rate (partial correlation coefficients)*

	Rest and exercise	Rest	Exercise
All data	0.36	0.46	0.18^{NS}
	(82)	(57)	(25)
All data excluding those with nicotinate	0.60	0.52	0.34^{NS}
	(62)	(47)	(15)

not significantly different from that in the presence of nicotinate (109.8 ± 2.6 ml/l) although the extraction of FFA is significantly lower in the presence of nicotinate. It is very likely however that there would be an extraction of FFA below which no further decrease in myocardial oxygen requirements could be observed.

The two other possibilities are that myocardial oxygen requirements determine myocardial FFA extraction and that a third factor determines both these extractions. The first of these cannot be excluded on the basis of the present series in vivo in man but it is not compatible with the animal work to which reference has already been made, and there is no reason to believe that increased oxygen consumption should increase FFA but not carbohydrate uptake.

Substrate and oxygen metabolism

The biochemical basis for an action of FFA on both myocardial carbohydrate extraction and oxygen requirements may be its oxidation to acetyl CoA units. An increase in the ratio of these units to free CoA may lead to the inhibition of pyruvate dehydrogenase (16) and, by increasing citrate concentration, inhibit phosphofructokinase (34). Both these inhibitions will slow down glycolysis and may thus lower glucose uptake. At the same time increased oxygen uptake may in part be related to oxidation of extra citrate without formation of ATP (8, 17).

Inhibition of pyruvate dehydrogenase will not be followed by an increased intracellular pyruvate concentration and, it is assumed, decreased pyruvate extraction unless pyruvate is still formed from glycolysis and/or lactate. The dependence of the relationship $C_{(a-cs)}$ pyruvate/$C_{(a-cs)}$ FFA on $C_{(a-cs)}$ glucose and on $C_{(a-cs)}$ lactate indicates this.

An interesting possibility is that FFA might affect myocardial metabolism in part indirectly through the displacement of thyroxine from plasma proteins. Hillier (19) has shown that the isolated perfused rat heart takes up more thyroxine at higher concentrations of FFA in the perfusate. However the dog heart in situ appears resistant to the uncoupling of oxidative phosphorylation by thyroxine (33). If this can be applied to the human heart, an effect of FFA on myocardial oxygen metabolism may not depend to any great extent on thyroxine.

During myocardial ischaemia the supplies of both oxygen and anaerobic fuel become critical. Since there is evidence that death after acute myocardial infarction is more frequent where plasma FFA concentrations are high (32) the present findings may prove of importance in the understanding and management of episodes of myocardial ischaemia.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Swedish Medical Research Council (No 19X-204). M. L. W. was recipient of a Life Insurance Medical Research Fund of Australia and New Zealand Overseas Research Fellowship and B. W. L. recipient of a Wellcome Swedish Travelling Fellowship.

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Acta med. scand. Vol. 193 pp. 97–101 1973

FIRST DEGREE HEART BLOCK AFTER DC COUNTERSHOCK

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Abstract During 2 / years 131 patients ith atrial arrhythmias hav been treated with DC countershock on 146 occasions. In 31 cases first degree heart block (AV-I) was observed after conversion to sinus rhythm. Different clinical parameters are discussed in relation to the development of postconversion AV I. Three out of four patients who developed circulatory arrest during the postconver sion period had had AV-I after conversion. Careful observation of patients who develop postconversion AV I is recommended.

Arrhythmias after DC countershock may be divided into two groups—those occurring immediately after the shock and those appearing after the conversion to sinus rhythm (5 12). The first type of arrhythmia has been considered relatively benign, while the second type more often leads to serious complications.

The importance of digitalis and/or quinidine therapy for the frequency of serious postconver sion arrhythmias has often been stressed (1 5 6 9 11 12).

From Jan. 1967 to June 1970 we have observed five cases of circulatory arrest in connection with DC countershock. One of these occurred immediately after the shock, thus belonging to the first group of arrhythmias mentioned above; it was of short duration (40 sec) and the patient recovered spontaneously The remaining four cases belong to the second group of postconver sion arrhythmias and required intensive therapy: these patients are presented separately They all had continuous digitalis therapy and three of them had also received quinidine prior to the conversion attempt. Another characteristic was that three of them developed a first degree heart block (AV I) after the conversion to sinus rhythm. This observation led us to examine the occur rence of and factors possibly related to post conversion AV I.

MATERIAL AND METHODS

From Jan. 1967 to June 1970 DC countershock treatment was carried out on 146 occasions at the Department of Medicine at Serafimerlasarettet. Eight of 131 patients (83 males and 48 females, ranging in age from 35 to 76 years) were treated more than once. On 130 occasions the patients had atrial fibrillation and on 16 occasions atrial flutter. The duration of the arrhythmia was less than 1 year in 40% of the cases. Thirty patients had VOC, mostly mitral valvular disease, such patients were treated on 37 occasions. T enty patients had hyper tension (diastolic BP > 100 mmHg) and 11 had had myocardial infarction. On 136 of 146 occasions (93) the patients were converted to sinus rhythm.

Among the patients converted to sinus rhythm there were only 3 who had not been treated with digitalis. Digoxin had been taken by 103 patients, digitoxin by 22 and scillaren by 4 4 patients were digitalized ith lanatoside C Digitalis was ithdrawn 1 7 days before the countershock treatment in 84 cases, hile in 49 cases therapy was continued. On 91 occasions quinidine in daily dosage of 1.2 g was administered 1 2 days prior to shock treatment.

Electrolyte balance was checked and corrected, if necessary and chest X-rays were taken. ECG was checked before, immediately before and immediately after the countershock treatment and on the following 4 days.

The patients ere premedicated with atropine and anesthetized with short-acting barbiturate. The DC countershocks are delivered by cardioverter from the American Optical Company

T determine statistical ignificance, the unpaired t-test or χ^2-test have been used. The significances have been expressed as follows. not significant $0.05 < p$ almost significant $0.01 < p \leq 0.05$, significant $0.001 < p \leq 0.01$ and highly significant $p < 0.001$.

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Table I. *Comparison between the patients with and without postconversion AV I*

	No. of episodes	Sex (♂)	(♀)	Mean age (y)	Atrial fibrillation	Atrial flutter	Hypertension	VOC	Myocardial infarction	Mean heart size (ml/m² BSA)	Mean serum potassium (mEq/l)	Mean serum calcium (mEq/l)
With AV-I	105	65	40	60	94	11	13	19	6	563	4.4	4.9
Without AV I	31	17	14	63	27	4	7	11	5	667	4.3	4.9
Total	136			$p<0.05$	121	15	20	30	11	$p<0.001$		

CASE REPORTS

Case 1

A 65-year-old female with diabetes, hypertension and atrial fibrillation since 3 years. Relative heart olume 680 ml/m² BSA. The patient had continuous digoxin and quinidine therapy prior to DC shock treatment, after which she converted to nodal rhythm. Drug therapy was continued. Several hours later on the same day she had circulatory arrest. After precordial blow nodal rhythm was registered. Atropine had no effect. On the following day the patient had sinus rhythm with AV I (P–Q 0..5 sec) and bigeminal nodal beats. Shortly after another atropine injection she developed ventricular tachycardia and fibrillation. She was defibrillated and converted to nodal rhythm ith intervening VEB. Lignocaine was gi en, but few hours later the patient again developed ventricular fibrillation and was converted to nodal rhythm which changed into atrial flutter and finally atrial fibrillation.

ase 2

A 74-year-old hypertensive female with atrial fibrillation after myocardial infarction 3 months earlier Relative heart olume 560 ml/m² BSA. She had continuous digoxin and quinidine therapy prior to DC shock. She was converted to sinus rhythm with AV I (P–Q 0.26 sec). Drug therapy was continued. A few hours later the patient had circulatory arrest and after transthoracic cardiac massage sinus rhythm with AV I (P–Q 0..8 sec) was registered. Shortly afterwards she had t attacks of ventricular tachycardia, which ere successfully treated with lignocaine. Sinus rhythm ith AV-I as registered once more and the AV-I persisted when the patient left hospital several days later

Case 3

A 52-year-old male with atrial fibrillation since 3 years. He had had a suspect myocardial infarction and the relative heart volume was 980 ml/m² BSA. On admission the patient was heavily incompensated and he was treated with digitalis and diuretics. No quinidine was given. Immediately after conversion the patient had nodal rhythm which spontaneously gave w y to sinus rhythm. About 6 hours later the patient had a circulatory arrest and was resuscitated. After that regular sinus rhythm was noted. However a few hours later the patient developed entricular fibrillation and was defibrillated to sinus rhythm.

Case 4

A 54-year-old female who had been operated on 5 years earlier for mitral stenosis and combined aortic valvular disease. Relativ heart olume 780 ml/m² BSA. She as treated with digoxin and quinidine before the con ersion attempt. She was electrically converted to sinus rhythm w th AV I (P–Q 0.24 sec). Drug therapy was continued as before and the P–Q interval was normal af.er 2 days. However because of sinus bradycardia and th. reappearance of AV-I (P–Q 0..3 sec) digitalis was ithdraw on the 4th day after conversion. On the following day the patient had a circulatory arrest and as resuscitated, after which atrial fibrillation was registered.

Three eeks later the patient was readmitted. No digitalis or quinidine treatment was given and the patient was electrically converted to sinus rhythm. Afterwards digitalis therapy as inhibited without complications.

RESULTS

The patients were converted to sinus rhythm by countershock treatment on 136 of 146 occasions. In 31 of the successful attempts an AV I was registered after the conversion ($P–Q > 0.22$ sec) in 14 of these cases the block disappeared within one day and in five more patients it disappeared in two days. Four patients were discharged from hospital with a persisting block after four days. In Table I a comparison is given between the groups with and without postconversion AV I.

The mean age in the group with AV I was almost significantly higher than in the other group. Sex distribution, hypertension frequency and types of pre-existing arrhythmias did not differ significantly There was no difference in the frequency of VOC or myocardial infarction.

The mean heart size on chest X ray before treatment was 667 ml/m² BSA in the group

Table II. *Preconversion treatment of patients with and without postconversion AV I*

	Total	Quinidine		Digoxin		Digitoxin		Scillaren	Lanatoside C	No digitalis
			%		%		%			
With AV-I	31	23	74	19	61	10	32	0	0	2
Without AV I	105	68	65	84	80	12	11	4	4	1
Total	136	91		103		22		4	4	3

with AV I as compared to 563 ml/m² BSA in the other group (highly significant difference). The mean values of serum potassium and calcium did not differ significantly

In the group with AV I 74% of the patients had been treated with quinidine as compared to 65% in the other group (difference not significant).

In the group with AV I 94% had been treated with digitalis compared to 99% in the group without AV I. Regarding the use of different digitalis drugs, it should be noted that 10 out of 31 patients in the AV I group had been taking digitoxin in the other group only 12 out of 105 patients had been given this drug (Table II).

In 84 cases digitalis was withdrawn prior to shock treatment, while in the remaining 49 cases therapy was continued. There was no significant difference between the time of digitalis withdrawal in the two groups (Fig. 1). In 10 out of 22 patients in the digitoxin group the drug was not withdrawn, seven of these patients developed postconversion AV I.

DISCUSSION

Postconversion AV I was recorded in 7% of the cases treated in a study by Resnekov and Mc Donald (8). In the present material the frequency was 23% However comparison between the two materials reveals important differences. The mean age in the present study was more than 60 years, while in Resnekov and McDonald's study 60% of the patients were less than 50 years of age. Furthermore, 68 out of 204 patients in the above material had no overall enlargement of the heart, whereas only 11 of 131 patients in our study had a relative heart size of 450 ml/m² BSA or less. The number of patients with VOC was higher and the number of patients with hypertension lower in Resnekov and McDonald's study than in the present one. Sex distribution and types of pre-existing arrhythmias were similar in the two materials. Differences in digitalis and quinidine treatment probably exist, but insufficient information for comparison is presented by the above authors. In a series of 148 patients 36 developed first degree heart block (24%) after conversion to sinus rhythm on high doses of quinidine (2). Sixteen of the 148 patients had circulatory arrest after conversion to sinus rhythm.

A comparison between the patients with and without postconversion AV I in the present study shows that only the heart size is significantly different and that the mean age is almost significantly higher in the AV I group. The differences in heart size and mean age between Resnekov and McDonald's study and the present one may to some extent explain the different frequences of postconversion AV I.

Of the patients with AV I 75% had been treated with quinidine as compared to 65% in the group without AV I the difference is not significant. Of 91 patients who had been treated with quinidine, 23 (25%) developed postconversion AV I. Among 45 patients who had not received quinidine treatment 8 (18%) developed AV I, the difference is not significant.

Only three patients out of 131 had not been treated with digitalis, two of whom had not received quinidine either These two patients had the longest postconversion P–Q times registered, 0.47 and 0.36 sec. These patients and two others are the only ones who have been discharged from hospital with persisting AV I. It should be noted that one of them had had AV I some years before atrial fibrillation was diagnosed.

With regard to digitalis therapy a difference was noted in the frequency of AV I between digitoxin- and digoxin-treated patients. The time of withdrawal did not influence the AV I fre-

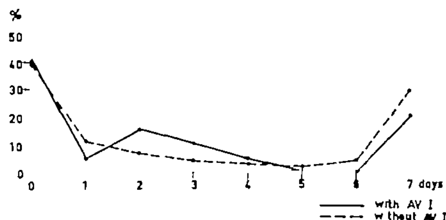


Fig 1 Comparison of digoxin withdrawal between patients with and without postconversion AV I.

quency in the digoxin group. In the digitoxin group the number of patients was too small to allow any conclusions to be drawn. Ten out of 22 patients in this group developed postconversion AV I, nine of whom also had quinidine therapy. Ten of the remaining 12 patients in the digitoxin group who did not develop AV I had not been taking quinidine. This difference in the frequency of AV I between the patients treated with digitoxin and quinidine and those treated with digitoxin only is highly significant. However the frequency of postconversion AV I in patients treated with digitoxin and quinidine did not differ significantly from the frequency in those treated with digoxin alone.

The four separately described cases of circulatory arrest after conversion had all been treated with digitalis. Three of them had also received quinidine treatment. All but one developed postconversion AV I, one of them after a period of nodal rhythm. The fourth patient was converted without any complications or AV I some weeks later: this time she had not been treated with digitalis or quinidine. The only patient with circulatory arrest who had not been treated with quinidine had the biggest relative heart volume in the whole study (970 ml/m² BSA). One of the patients (no. 2) had a history of AV I in connection with acute myocardial infarction. The patient who had the longest P–Q time recorded, but who had no serious complications, also had a history of AV I. Among 105 patients who did not develop postconversion AV I there was none with a history of AV I.

Gilbert and Cuddy (3) reported a high frequency of postconversion AV I in a small group of patients who had all been treated with digitalis and, in most cases, with quinidine as well (P–R > 0.21 sec in 46% P–R > 0.22 sec in 25%). Two patients had developed ventricular fibrillation a few hours after conversion and could not be resuscitated. One of them had multifocal VEB before and after conversion and also a postconversion P–R time of 0.22 sec. Postconversion circulatory arrests in patients who have had multifocal VEB prior to conversion have been described (10). Treatment with DC countershock in patients with these types of arrhythmia has been avoided in the present material.

It is well known that digitalis and quinidine prolong the AV conduction time. It also seems that DC countershock itself may produce a prolongation of the AV conduction time. Thus it is not surprising that a combination of all these factors may produce a high frequency of AV I.

Lown et al. (7) measured the P–R interval in 45 patients restored to sinus rhythm by DC shock. They also noticed a high incidence of AV I. They considered that the prolongation in AV conduction time was not due to the procedure of reversion itself. In contrast to our findings they found that in the majority of the patients the prolonged postconversion P–R interval persisted. In only four of our 31 patients with AV I the block was still present after four days. Three of our four patients with circulatory arrest had had postconversion AV I, i.e. they make up 10% of all patients who developed AV I. In a follow-up

study of 138 patients who had been converted to sinus rhythm by DC countershock Korsgren et al. (4) describe three patients who suddenly expired shortly after being discharged from hospital. One of them had a history of AV I and one developed AV I after conversion.

It is our opinion that patients who develop AV I after DC countershock should be carefully observed and that digitalis and quinidine should be temporarily withdrawn or administered with caution. A history of AV I should not be overlooked.

ACKNOWLEDGEMENT

This study was supported by grant from the Swedish National Association against Heart and Chest Diseases.

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THE MECHANISM OF MYOCARDIAL INFARCTION FOLLOWING PROSTHETIC AORTIC VALVE REPLACEMENT

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Abstract. During a 5-year period 42 of 243 patients, subjected to prosthetic aortic valve replacement at the University Hospital in Oslo, died. Nine of these patients died from myocardial infarction. The mechanism of myocardial infarction in these patients has been studied by correlation between clinical, ECG, coronary angiographical and autopsy studies. Two patients died from thrombosis on the artificial valve extending into a coronary artery, one from coronary embolism (?), two from surgical interference with the coronary circulation. In two patients the myocardial infarction was related to coronary atheromatosis. In the last two patients the coronary arteries were normal. In both these patients there was extensive subendocardial hemorrhage and myocardial necrosis. In order to prevent myocardial infarction in the postoperative period it is important to monitor these patients for arrhythmias and to prevent postoperative hypotension. Anticoagulant treatment should be carried out with strict supervision of its efficacy. Agents to prevent platelet thrombosis should be evaluated in these patients with high risk of thrombus formation.

Myocardial infarction is one of the leading causes of death in the postoperative period in patients with heart valve replacements (1, 2, 3, 4, 7, 9, 10, 12, 15, 16). The pathogenesis of myocardial infarction in these patients is varied. It may be caused by coronary embolism (1, 5, 8). It may be due to thrombus formation on the artificial valve extending into a coronary artery. Cannulation of a coronary artery during operation may produce injury to the artery with subsequent intimal dissection (6). Surgery itself may cause injury of coronary arteries due to mechanical interference of the prosthesis (16) or coronary blood flow may be compromised by sutures (16). Many of these patients are in the higher age group and it is therefore not surprising that they may have coronary occlusion on an atherosclerotic basis in the postoperative period (3, 4, 11). Myocardial infarction has, however, been observed at autopsy in patients with normal coronary arteries, where the pathogenesis of the infarction is difficult to explain (3, 4, 9, 12, 16). In some of these patients autopsy has demonstrated patchy myocardial necrosis or extensive subendocardial hemorrhage.

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MATERIAL

During the period 1965–70, 243 patients were subjected to aortic valve surgery with implantation of a prosthetic valve in this hospital. In the postoperative period there were 42 deaths. Eight of these were caused by myocardial infarction, one further patient died 2 months following the operation (Table I). During the same period myocardial infarction was diagnosed in six surviving patients.

RESULTS

All the nine patients who died from myocardial infarction were men. Their age ranged from 33 to 61 years. In the postoperative period the clinical history may be difficult to obtain and evaluate as most of the patients have fever and chest pain following the operation. Frequent ECG observations are important in this period to detect arrhythmias and signs of myocardial injury. In the later postoperative period these patients should be treated in hospital to combat complications which may arise. Such ECG and clinical observations have made it possible to fix the date of the myocardial infarction in eight of our patients. In five of the patients the infarction occurred

Table I. *Cause of death in 62 of 243 patients subjected to aortic valve replacement*

	Operative	Late	Total
Arrhythmia	12	0	12
Myocardial failure	11	3	14
Sepsis infection	7	6	13
Myocardial infarction	8	1	9
Embolism	1	4	5
On the table	2	0	3
Aortic rupture	0	1	1
Unknown	0	4	4
Carcinoma	0	1	1
	42	20	62

on the first postoperative day (Table II) In one patient (no. 2) the myocardial infarction was undiagnosed. This patient had cerebral injury with coma following the operation. He had to be treated with a respirator and did not regain consciousness before death. He also had atrioventricular block during the operation, which necessitated implantation of a pacemaker Only two patients died during the first few hours following the infarction. The other patients survived within 1–21 days.

Angina pectoris is one of the leading symptoms in patients with aortic valvular disease. In our material angina pectoris was observed in 49% of the patients. It may be functional or may be caused by anatomic changes of coronary arteries. Coronary angiography is carried out preoperatively in these patients to evaluate the coronary circulation with regard to operability and to surgical cannulation during operation. Four of the nine patients who died from myocardial infarction had angina pectoris preoperatively Coronary angiography demonstrated occlusion of the right coronary artery in one of the patients (no. 5) and stenosis of the circumflex branch of the left coronary artery in another (no. 6). The other seven patients had patent coronary arteries on coronary angiography (Table II)

Table II. *Data on the 9 male patients who died from myocardial infarction (MI)*

Pat. no.	Age (y.)	MI day	Death day	Coronary angiography
1	33	1	2	Patent
2	53	?	10	Patent
3	59	17	17	Patent
4	46	60	60	Patent
5	52	1	16	Occl. cor art.
6	61	13	17	Stenos. circumflex art.
7	49	1	6	Patent
8	51	1	21	Patent
9	48	1	13	Patent

Following myocardial infarction ECG supported the diagnosis in seven of the patients; four of them showed a pattern of posterior wall infarction and three a pattern of anterior or anteroseptal infarction. In one patient (no. 2) pacemaker implantation made the ECG diagnosis impossible In another patient (no. 4) ECG showed ventricular fibrillation from which the patient died (Table III)

Autopsy studies are important to determine the mechanism of myocardial infarction in these patients. Autopsy has been carried out in all our patients (Table III). In two of them (nos. 1 and 2) the coronary arteries were patent. A more detailed report of these patients is given below Two patients (nos. 3 and 6) had a thrombus formation on the artificial valve with extension of the thrombus into the left coronary artery with resulting anterior wall infarction. In these patients the myocardial infarction occurred on the 17th and 13th postoperative days, respectively Both patients were treated with anticoagulants, but the prothrombin time was not in the effective therapeutic range at the time when the myocardial infarction occurred. The thrombotest percentage was 40% in patient 3 on the day of infarction and 35% in patient 6. In one patient (no. 6) who died suddenly on the 21st postoperative day autopsy showed only minimal coronary atheromatosis, but there was a thrombus occluding the descending branch of the left coronary artery (coronary embolism?) This patient was treated effectively with anticoagulants. The prothrombin time on the day of death was 7% thrombotest.

In two patients there was surgical interference with the coronary circulation. In patient 7 the prothesis interfered with the coronary circulation producing extensive myocardial infarction. In patient 9 postoperative bleeding necessitated suture of the descending branch of the left coronary artery which resulted in anterior wall myocardial infarction.

In two patients myocardial infarction appar

Table III. *ECG and autopsy findings*

Pat. no.	ECG	Infarct	Cor. arteries	Complications
1	Post inf. ventr. tachyc.	Postero-lateral	Patent	Septal bleeding
2*	AV block	Ant. & post.	Patent	Septal bleeding
3	Ant. inf.	Anteroseptal	Thrombus left cor. art.	Coma, resp. treatment
4	Ventr. fibr.	Anterior	Occl. ant. desc. art.	—
5	Post inf.	Posterior	Occl. right cor. art.	— (undiagn.)
6	Anteroseptal inf.	Anterior	Thrombus left cor. art.	—
7	Post inf.	Total	Occl. by ball valv.	—
8	Post inf.	Apex	Occl. ant. desc. art.	—
9	Ant. inf.	Anterior	Suture ant. desc. art.	—

* Pacemaker

ently was related to coronary atheromatosis. One of the patients (no. 5) had previously had a posterior myocardial infarction in 1965 and coronary angiography demonstrated occlusion of the right coronary artery. In this patient there was further occlusion of this artery following the operation, with death on the 16th postoperative day. In one patient (no. 4) who died suddenly on the 60th postoperative day from ventricular fibrillation, autopsy demonstrated occlusion of the descending coronary artery near the apex with myocardial necrosis. The coronary arteries were otherwise patent.

CASE REPORTS

Case 1

This patient was operated upon on Feb. 25, 1969, for severe aortic stenosis and mitral insufficiency with implantation of Starr-Edwards valve in the aortic ostium and Beall valve in the mitral ostium. Postoperatively there was hypotension and ventricular tachycardia (Fig. 1). Treatment with lidocaine 60 mg i.v. stopped the tachycardia and the ECG showed AV block, grade I, and inferior wall infarction. Later on there was atrial fibrillation. The patient died on the second postoperative day. At autopsy the heart was found to be considerably enlarged (1 100 g) with thickening of the walls of both ventricles (left 2 cm, right 0.8 cm). The inner surface of the left ventricle showed diffuse hemorrhage in the upper part of the septum, partly also in the anterior wall, with small areas of fibrosis in the latter region. The artificial valves seemed to fit well, with only minor reactive changes in the area. Coronary angiography and later inspection revealed no stenoses and only insignificant atherosclerosis. Microscopically there was extensive hemorrhage in the left ventricular and septal myocardium with recent myocardial necrosis and patchy myocardial fibrosis (Fig. 2).

Case 2

This patient was operated upon on June 18, 1969 for aortic insufficiency and mitral stenosis and insufficiency with transplantation of Starr-Edwards prosthesis to the aortic ostium and Beall prosthesis to the mitral ostium. During the operation AV block appeared and an epicardial fixed-rate pacemaker was therefore implanted. He did not regain consciousness following the operation and had to be treated with respirator until he died on the 10th postoperative day. Postoperative ECG showed fixed-rate pacemaker rhythm. At autopsy there was subendocardial hemorrhage in the upper part of the ventricular septum and in the posterior wall of the left ventricle near the septum. The coronary arteries were patent on inspection and on coronary angiography (Fig. 3). Microscopically there was extensive myocardial necrosis both in the anterior and posterior wall of the left ventricle and hemorrhage in the ventricular septum. (Fig. 4).

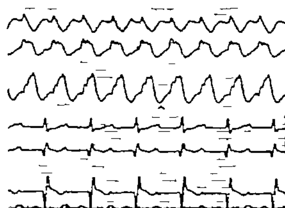


Fig. 1. ECG from patient 1 on the first postoperative day. Upper ECG shows ventricular tachycardia. Following 0.06 g lidocaine the ECG shows AV block grade I and inferior wall infarction.



Fig 2 Patient 1. Left ventricular myocardium. Myocardial fibrosis and hemorrhage with recent myocardial necrosis. Hematoxylin eosin stain × 63.

DISCUSSION

The mechanism of myocardial infarction in patients with prosthetic heart valves is varied. In our material there were thus 2 instances of thrombus formation on the artificial valve with extension into a coronary artery. In two patients the coronary occlusion occurred on the basis of pre existing coronary atheromatosis. In one patient coronary embolism is supposedly the cause of coronary occlusion. In two patients there was surgical interference with the coronary circulation. In the last two patients the coronary arteries were patent, but there was extensive subendocardial hemorrhage. The mechanism of the bleeding with concomitant myocardial necrosis is disputed. Some of the patients, like patient 1 postoperatively had hypotension and ventricular tachycardia, which may contribute to myocardial necrosis. Other contributory causes may be cross clamping of the aorta, antifoam or calcific embolism or platelet thrombosis in small blood vessels (12). In some patients with postoperative myocardial infarction microscopic calcifications may be found in the myocardium (16). Ventricular fibrillation and postoperative treatment with pressor agents have been considered as important contributory factors in left ventricular hemorrhagic necrosis (14).



Fig. 3. Post-mortal coronary angiography from patient 2 demonstrating Starr-Edwards prosthesis in the aortic ostium and Beall prosthesis in the mitral ostium. The coronary arteries are patent.

The most important cause of myocardial infarction in our material is thrombus formation. All our patients were treated with anticoagulants to prevent thromboembolism. In spite of this, thrombus formation still occurred. In two of the patients (nos. 3 and 6) there was thrombosis on the artificial valve with extension into a coronary artery. The anticoagulant treatment had not been effective enough, as the prothrombin time was outside the therapeutic range when the myocardial infarction occurred. In a third patient (no.



Fig. 4. Patient ... Left ventricular wall near the apex. Myocardial necrosis. Hematoxylin eosin stain 63.

8) with embolism to a coronary artery the source of the embolus was not discovered and his prothrombin percentage was in the low therapeutic range. Anticoagulants are known not to prevent platelet thrombosis, which is the initiating part of thrombus formation in arteries. Treatment with agents which may prevent platelet aggregation, like acetylsalicylic acid and persantin (17), should therefore be considered. Preliminary studies to evaluate the effect of these agents in patients with prosthetic heart valves are in progress in our department (13).

Preventive measures to be taken are otherwise close supervision during the immediate post operative period. It is especially important to prevent hypotension and arrhythmias and to treat these complications vigorously when they arise.

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Acta med. scand. Vol. 193 pp. 109–112, 1973

AZATHIOPRINE TREATMENT IN ACTIVE CHRONIC HEPATITIS

Evaluation of Dose Levels

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Abstract. A retrospective study of 29 patients with active chronic hepatitis treated over 38 different periods with azathioprine was carried out with particular attention to the effects of high (above 1.5 mg/kg) and low (below 1.5 mg/kg) doses. Early side-effects (within 4 weeks) occurred in 7 of 10 patients using above 2 mg/kg and in 6 of 11 patients on 1.5–2 mg/kg azathioprine. Of the patients treated with the low azathioprine dose only 4 of 17 had early side-effects. Eighteen patients were treated up to 2 years, among whom 2 of 12 on low dose and 3 of 6 on high dose had late side-effects. Of 9 patients treated for more than 2 years (average 2 3/4 years) on low azathioprine dose late side-effects occurred in 2. The clinical course of the liver disease in these patients showed that 5 of 8 treated on high dose were unchanged or improved compared to 15 of the 21 on the low dose. Serum γ-globulin and SGPT decreased significantly in the patients on the low azathioprine dose concomitantly with a non-significant increase in serum albumin. These changes were not less marked on low than on high dose. Our experiences thus favor doses below 1.5 mg/kg when azathioprine is used in active chronic hepatitis.

Corticosteroid therapy increases life expectancy in active chronic hepatitis (1) and cirrhosis of the liver (2, 10). Immunosuppressive drugs may also be of value in chronic liver disease, particularly in active chronic hepatitis (3, 4, 5, 7, 8), but controlled studies have not yet appeared comparing the effects of such drugs alone or combined with corticosteroids with the effects of steroids alone. A controlled study presenting the benefit of azathioprine treatment in primary biliary cirrhosis has recently appeared (9).

Questions to be answered in the future are not only indications for immunosuppressive therapy but also its duration and the dose to be used. Hazards are attached to the use of azathioprine and doses of more than 100 mg/day have been warned against because they may be followed by increased jaundice and development of hepatic coma (3, 4, 5).

We have in retrospect studied 29 patients with active chronic hepatitis treated over 38 different periods with azathioprine during the last 4 years and paid particular attention to the side-effects and the beneficial effects on high (above 1.5 mg/kg) and low (below 1.5 mg/kg) doses. Our experiences seem to indicate that there are fewer side-effects on the smaller dose but at least the same clinical and biochemical benefit.

PATIENTS AND METHODS

At Medical Department A, University Hospital, Rikshospitalet, Oslo, we have treated altogether 29 patients, 20 women and 9 men, with active chronic hepatitis with azathioprine during the years 1967–71. Their age varied from 15 to 69 years—average 29 years. The average duration of liver disease before azathioprine treatment was 4.4 years. Diagnosis was based on clinical history, biochemical findings and on the histological criteria for chronic hepatitis laid down by a group of European pathologists as described by de Groote et al. (6). Three of the patients also had ulcerative colitis, and in 1 the disease was most likely induced by an oxyphenisatine laxative. The 29 patients were treated in altogether 38 periods with azathioprine. Additional prednisone was given during 33 of these periods. Seven patients were given treatment in 2 periods and 1 patient in 3 periods. The drug used was Imurel®.

RESULTS

Side-effects during first 4 weeks of azathioprine treatment

The following criteria were used for the definition of early side-effects: anemia—fall in Hb

Table I. *Side-effects during first 4 weeks of treatment*

	Azathioprine dose (mg/kg)		
	>2	1.5–2	<1.5
Total no. of pats.	10	11	17
Prednisone-treated	9	10	14
Anemia	3	1	1
Leukopenia	2	2	1
Thrombopenia	2	2	1
Bilirubin incr.	4	3	2
SGPT incr	1	2	2
Fever	1	0	0
Sepsis/infection	0	0	0
Gastroint. compl.	1	1	0
Mors	0	1	0
Total no. of pats. with side-effects	7	6	4

of more than 1 g/100 ml, leucopenia—fall to less than 3 000/µl thrombocytopenia—fall to less than 100 000/µl, bilirubin increase of more than 1 mg/100 ml and increase of SGPT of more than 50 %.

Table I demonstrates the early side-effects during 38 treatment periods.

Anemia. On the highest azathioprine dose Hb fell in 3 patients from 1.6 to 4.8 g/100 ml, in the dose group 1.5–2 mg/kg Hb fell 1 3 g/100 ml 1 and in the lowest dose group 1 1 g/100 ml in patient

Leucopenia. In all 3 dose groups total white counts in the registered patients with leucopenia were between 1 700 and 2 500/µl.

Thrombocytopenia. Counts ranged from 38 000 to 91 000/µl.

Table II. *Late side-effects in patients treated up to 2 years*

	Azathioprine dose (mg/kg)	
	1.5–2.5	<1.5
Total no. of pats.	6	12
Prednisone-treated	6	12
Fever	1	0
Sepsis	2	0
Other infection	1	1
Other complications	1	0
Mors due to side-effects	1	1
Mors due to liver dis.	0	3
Total no. of pats. with side-effects	3	2

Gastro-intestinal complications were duodenal ulcer with bleeding and gastritis.

It is seen from Table I that the total number of patients with side-effects is smaller in the group using the lowest azathioprine dose (4 of 17) than in the higher dose groups (7 of 10 and 6 of 11 respectively).

Late side-effects in patients treated up to 2 years

Table II shows side-effects occurring after the first 4 weeks in patients using azathioprine up to 2 years. The average period of treatment of the lowest dose was 8 months, and in the higher dose group 6 months. The average "low" dose was 62.5 mg/day and the average "high" dose 108 mg/day Since anemia, leucopenia and thrombocytopenia may result from hypersplenism secondary to the liver disease, these are not here registered as true drug side-effects. The infections were represented by abscess, urinary tract infections and furunculosis.

Side-effects leading to death were pancytopenia and agranulocytosis in 1 patient on high azathioprine dose. She had previously used azathioprine, but was on myleran when she died. The patient who died on low dose developed liver failure, probably due to the drug.

Of the 3 patients on the low dose who died due to the liver disease per se 2 went into final liver failure with hepatic coma a year after azathioprine was stopped, and 1 developed a hepatoma.

It is seen that the total number of patients with side-effects was 2 out of 12 on the low dose and 3 out of 6 on the high dose.

Late side-effects in patients treated from 2 to 5 years

Altogether 11 patients were treated from 2 to 5 years with azathioprine, the average treatment period being 2 3/4 years.

Table III demonstrates the side-effects in these patients. Two patients of the 9 on low dose (average 50 mg/day) and 1 of the 2 on high dose were registered as having side-effects.

Clinical evaluation and liver function

The effects of azathioprine treatment are presented as a total clinical evaluation and by analyzing biochemical data from the liver function tests.

Table III. *Late side-effects in patients treated 2–5 years*

	Azathioprine dose (mg/kg)	
	1.5–2	*<1.5*
Total no. of pats.	2	9
Prednisone-treated	1	5
Fever	1	0
Infection	1	0
Sepsis	1	1
Other complications	0	1
Mors	0	0
Total no. of pats. with side-effects	1	2

Table IV. *Total clinical evaluation*

	Azathioprine dose (mg/kg)	
	1.5–2.5	<1.5
Duration of treatment 1 mo.–2 y		
Worse	3	5
Unchanged	1	2
Improved	2	5
Duration of treatment 2–5 y (aver. 2 3/4 y.)		
Worse	0	1
Unchanged	1	0
Improved	1	8

Table IV presents the total clinical evaluation of the course of the disease. It is seen that 3 of 6 treated on high dose for an average period of 6 months were unchanged or improved, whereas 7 of the 12 on the lowest dose treated for an average of 8 months were unchanged or improved.

Of the 9 patients treated for more than 2 years (average 3 3/4 years) on low dose the improvement occurred in 8.

Liver function tests are presented in Table V which reveals a significant decrease in γ-globulin and SGPT in the patients treated on the low azathioprine dose and also a non-significant increase in serum albumin concentration. In 4 patients who died billirubin increased in 3.

The fall in serum γ-globulin and SGPT and the increase in albumin were not less marked on the low dose than on the higher doses.

DISCUSSION

This retrospective analysis demonstrates that azathioprine in doses less than 1.5 mg/kg leads to less side-effects in the initial treatment period than higher doses when used in active chronic hepatitis. In the further course a similar trend is noted, but the figures are too small to allow conclusive statements. Azathioprine in low dose (under 1.5 mg/kg), furthermore seemed not to be inferior to the higher doses as judged from the total clinical evaluation and from the improvement in the registered liver function tests. In total 15 of 21 on low dose were unchanged or improved compared to 5 of 8 on high dose.

Table V. *Liver function tests (treatment 1 mo.–2 y.)*

	Improved	Same or worse	Mean before	±S.D.	Mean after	±S.D.	Significance of difference
Dose <1.5 mg/kg (12 pats., mean treatment 8 mo.)							
Albumin (g/dl)	11	1	2.82	0.55	3.23	0.71	n.s.
γ-globulin (g/dl)	11	1	3.74	0.95	2.71	0.73	<0.01
SGPT (U/l)	10	2	236	195	72	91	<0.01
Bilirubin (mg/dl)	8	0	8.9	9.4	1.6	1.03	<0.05
In the pat. who died	1	3	8.4		25.7		
Alk. phosph. (U/l)	8	4	116	59	83	54	n.s.
Dose 1.5–2.5 mg/kg (6 pats., mean treatment 6 mo.)							
Albumin (g/dl)	4	2	3.06	0.20	3.17	0.48	n.s.
γ-globulin (g/dl)	4	2	3.78	0.69	2.77	0.90	<0.05
SGPT (U/l)	4	2	251	158	94	80	<0.05
Bilirubin (mg/dl)	5	1	1.82	0.67	1.22	0.84	n.s.
Alk. phosph. (U/l)	4	2	202	71	117	86	

Table I. *Enzyme determinations correlating with the histological findings in the whole patient group and in some groups with liver disease*

	No. of pats.	No. of correlating enzyme values Urocanase	SGOT	SGPT
Whole group	186	134	148	144
Liver cirrhosis	13	9	11	12
Acute hepatitis	27	24	23	18
Chronic hepatitis	15	12	13	10
Cholestasis	7	5	3	3
Liver metastases	7	6	5	6

Table II. *Number of patients, in whom no correlation between the histological examination of the biopsies and the serum levels of urocanase, SGOT and SGPT was found, divided according to presence or absence of liver cell necrosis*

Necrosis	Urocanase	SGOT	SGPT
Present	9	13	20
Not present	43	25	22
Total	52	38	42

most of the cases different stages of the necrotizing process were present together, some of the following abnormalities were also observed: polymorphism of cells and nuclei, granular or vacuolated cytoplasm, mononuclear infiltration, development of Councilman bodies, and local disorganization of the trabecular structure.

Urocanase was measured according to Burobin et al. (2) with a slight modification (1). The serum samples were determined immediately or kept at −20°C for only a few days, because storage at −20°C for longer than 1 week was accompanied by a loss of enzymatic activity. In sera of 18 normal control subjects no urocanase could be detected.

SGOT and SGPT were measured as described by Reitman and Frankel (5) and expressed in Wroblewsky units. Taking the mean increased by 2 S.D., values below 50 U were considered to be normal.

In each case the enzyme levels were compared with each other and with microscopically ascertained liver cell necrosis. Statistical analysis was done with a four fold contingency table and Kendall's rank correlation test, with a double-tail probability: the level of significance was taken at $\alpha < 0.05$

RESULTS

The results are presented in Tables I and II and in Figs. 1–7. In the whole group of 186 patients necrosis of liver cells in the biopsies was

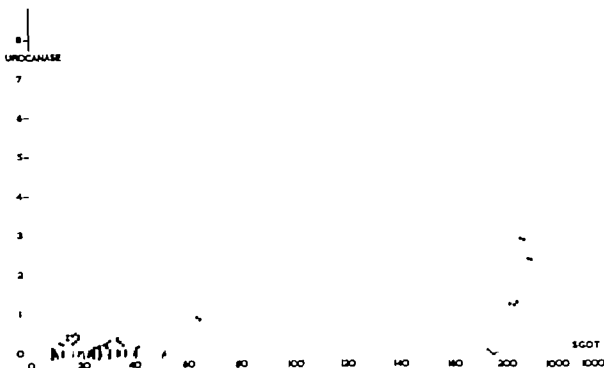


Fig. 1. Relation between urocanase and SGOT in the whole patient group. ● = liver cell necrosis present, ○ = not present.

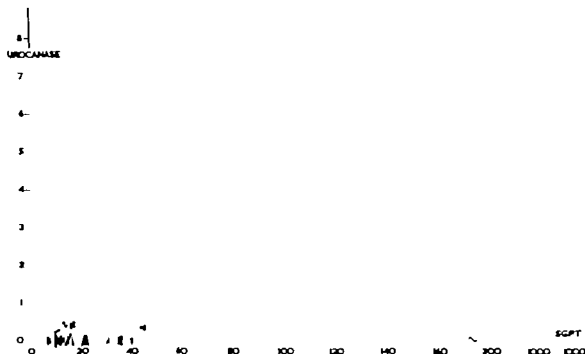


Fig. 2. Relation between urocanase and SGPT in the whole patient group. Symbols as in Fig. 1.

found in 52. Good agreement with the histological findings was found for urocanase levels in 134 patients, for SGOT levels in 148 and for SGPT levels in 144 patients. The number of correlations for these 3 enzymes were not significantly different when compared with each other ($p > 0.05$).

The number of patients in whom no correlation between the enzyme levels and the presence of liver cell necrosis was found is given in Table II. It is striking that non-correlating urocanase values are most frequently found in biopsies

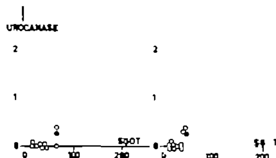


Fig. 3. Relation between urocanase, SGOT and SGPT in cirrhosis of the liver. Symbols as in Fig. 1.

from patients without necrosis. This difference proved to be significant ($p < 0.05$). In contrast, the number of SGOT and SGPT levels not correlating with the biopsy findings was not clearly different in the groups with or without liver cell necrosis ($p > 0.05$).

In patients with cirrhosis of the liver a correlation with the histological picture was found in 9 for urocanase, in 11 for SGOT and in 12 for SGPT. The number of correlations for urocanase was significantly lower than for SGOT and SGPT ($p < 0.05$) between SGOT and SGPT no difference of this kind was found ($p > 0.05$), (Table I and Fig. 3).

Twenty-four of the urocanase levels in the group with acute viral hepatitis correlated with the results of the biopsy. For SGOT and SGPT these numbers were 23 and 18, respectively. If compared with each other no significant differences between the numbers of correlations for urocanase, SGOT and SGPT were found ($p > 0.05$), (Table I and Fig. 4).

In chronic hepatitis correlations were found in 12, 13 and 10 cases for urocanase, SGOT and SGPT respectively. The number of correlations

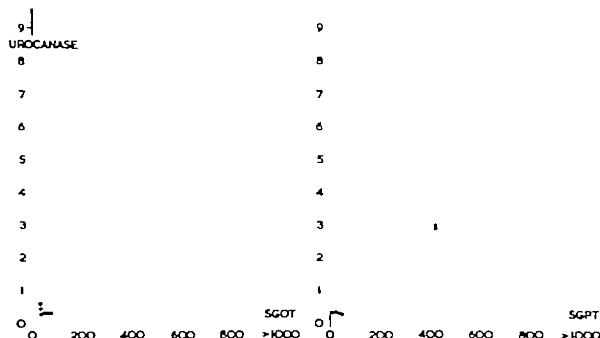


Fig. 4. Relation between urocanase, SGOT and SGPT in acute viral hepatitis. Symbols as in Fig. 1.

was lower for SGPT with respect to urocanase and SGOT ($p < 0.05$). For urocanase and SGOT no difference between the number of correlations could be calculated ($p > 0.05$), (Table I and Fig. 5).

The patient group with cholestasis showed correlations between the enzyme levels and the presence of cell necrosis in 5, 3 and 3 cases for urocanase, SGOT and SGPT respectively. These numbers are not significantly different ($p > 0.05$), (Table I and Fig. 6).

In liver metastases a correlation with the microscopic analysis was seen in 6 patients for urocanase, in 5 for SGOT and in 6 for SGPT. No significant differences between these numbers could be calculated ($p > 0.05$), (Table I and Fig. 7).

From the remaining patients no groups could

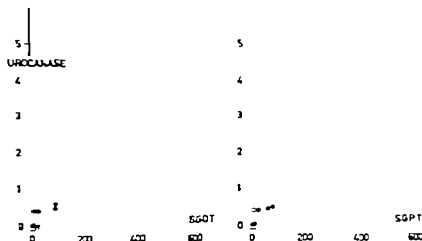


Fig. 5. Relation between urocanase, SGOT and SGPT in chronic hepatitis. Symbols as in Fig. 1.

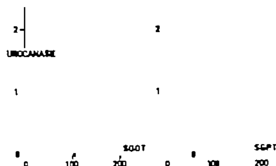


Fig. 6. Relation between urocanase, SGOT and SGPT in cholestasis. Symbols as in Fig. 1.

be composed suitable for statistical analysis because of the heterogeneity of their diseases.

Positive correlations between the absolute values for the 3 enzymes were found in the whole group and in the subgroups with chronic hepatitis, acute viral hepatitis, and liver metastases ($p < 0.05$). In the group of cirrhotic patients a positive correlation was only found between SGOT and SGPT values ($p < 0.05$). In the cholestatic group there was no correlation at all ($p > 0.05$).

DISCUSSION

These results suggest that there are no distinct differences between the means for determination of urocanase, SGOT and SGPT in serum to discern visible liver cell necrosis. In the groups with specific liver disease a few differences were found, but they did not point in the same direction. In patients with cirrhosis the number of correlations between the presence or absence of necrosis on the one hand and increased or normal enzyme levels on the other was lower for urocanse than for SGOT and SGPT. In chronic hepatitis, however, results of SGPT determinations were less correlated with cell necrosis. In the three other groups of patients the value of the three types of enzyme studies was not clearly different. That none of the enzyme determinations studied is clearly superior to the other two is most evident from the results in the whole patient group, listed in Table I, where no differences could be demonstrated.

It is remarkable that the non-correlating enzyme determinations are equally divided between the biopsies with or without necrosis for SGOT and SGPT but that non-correlating urocanase measurements are more frequently seen in biopsies without necrosis. This finding suggests that false positive results are relatively common in urocanase determinations, though one reason may be the rigid criteria we used for the presence of liver cell necrosis. It is certainly not excluded that cell injuries of minor degree may result in an escape of some urocanase from the damaged liver cells. This hypothesis is supported by the fact that the correlation between the absolute values of the enzymes is much better than the

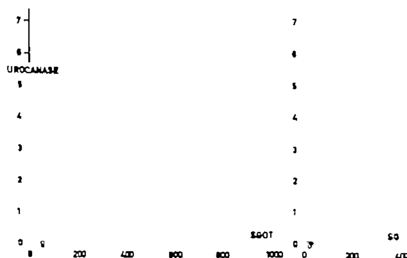


Fig. 7. Relation between urocanase, SGOT and SGPT in liver metastases. Symbols as in Fig. 1.

correlations of their levels with the presence or absence of cell necrosis.

An explanation of the disappointing degree of correlation between the serum levels of all three enzymes and liver cell necrosis might be that a blindly taken needle biopsy is not necessarily representative of the whole liver. There is no doubt that this possibility has to be considered, but it seems unlikely that it can be held responsible for all instances of missed correlations. In most of the cases in which no correlation could be found, this concerned only one of the enzymes. If the biopsy is at fault, a deviation of the result of all enzyme determinations has to be expected. We feel that both possibilities may occur: non-representative biopsies and non-correlating enzyme levels.

The determination of urocanase is a fairly time-consuming procedure. Another drawback of this method is the decreasing activity of the enzyme with storage of the serum samples for more than one week, even at −20 C. The combination of these two disadvantages makes the urocanase test unsuitable as a routine laboratory procedure.

In conclusion we feel that the determination of urocanase levels in serum has no advantage over the measurement of transaminases in the laboratory screening of liver disease, nor in ascertaining the presence of liver cell necrosis. However, in circumstances in which the origin of elevated transaminase levels is not clear, this test may be helpful to ascertain the presence of liver damage.

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Acta med. scand. Vol. 193 pp. 119–131 1973

BUMETANIDE, A NEW POTENT DIURETIC

A Clinical Evaluation in Congestive Heart Failure

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Abstract. Bumetanide, a new compound with diuretic action, has been evaluated in 106 patients with congestive heart failure. Bumetanide was found to be a very potent natriuretic and diuretic agent with a rapid onset and a short duration of action. Bumetanide natriuresis was associated with an increased urinary output of potassium and chloride and a trend to development of hypokalaemia, hypochloraemia and metabolic alkalosis. Bumetanide was able to decrease renal-diluting and renal-concentrating abilities and has apparently a site of action in the ascending limb of the loop of Henle. In comparative short-term and long-term studies bumetanide proved to be equipotent with furosemide at one-fortieth of its molar dosage. On continued daily administration for several months and with supplements of potassium chloride or spironolactone, bumetanide produced effective diuresis with minimal changes in blood chemistry, haematological status or liver function. Like other saluretics it caused a tendency to hyperuricaemia, but it did not provoke gouty arthritis or diabetes mellitus. Bumetanide is very well tolerated and is one of the most potent diuretics available to-day.

Bumetanide (3-n-butylamino-4-phenoxy-5-sulfamylbenzoic acid) (Fig. 1) is a new and potent diuretic compound which differs chemically from other diuretics (11, 12, 13).

A clinical evaluation of bumetanide as an oral diuretic agent in patients with congestive heart failure is the subject of this report.

PATIENTS AND METHODS

One hundred and six adult patients with evidence of organic heart disease and two normal volunteers were studied. The clinical diagnoses were as follows: arteriosclerotic heart disease 33, mitral valvular disease 24, aortic valvular disease 20, cardiomyopathy 10, congenital heart disease 8, hypertensive heart disease 4, cor pulmonale 4, and constrictive pericarditis 3 patients. All patients exhibited abnormal renal sodium and water retention, and most of them had received digoxin and diuretics previously.

Short-term studies

The patients received a low sodium diet, 5 g sodium chloride, 1 500 ml water and a supplement of 3 g potassium chloride daily. The subjects were observed for 2 or 3 days without diuretic treatment, while digoxin medication was continued unaltered. The following parameters were followed: 24-hour urinary volume, electrolyte and creatinine excretion and urinary osmolality. B.wt., serum electrolytes, creatinine, osmolality and Hb were measured every morning in the fasting state. The short-term studies were performed in the hospital while the patients were up and about and included the following treatment programmes.

(*a*) In 24 patients the 24-hour effect of bumetanide was measured as incremental changes in urinary excretion of sodium, potassium and chloride by subtracting the mean amount excreted in 24 hours during control days from that excreted on the day of drug administration. At the same time the 24-hour b.wt. change was registered. The material consisted of four groups of six patients who according to estimated requirements were given 1, 2, 3 or 4 mg bumetanide, respectively.

(*b*) *In 48 patients the* 24-hour effect of bumetanide was compared to that of furosemide and of placebo tablets by help of the rotation programme shown in Table I. Since the effect of a diuretic varies according to the pathophysiological status of the patient, the day of administration and the compound administered, the patients were allocated randomly to the dosage schedule in order to reduce the variance (18, 28, 30). The series comprised four groups of 12 patients who were given 1, 2, 3 or 4 mg *bumetanide and 40, 80, 120 or 160 mg furosemide*, respectively. The choice of dose levels for comparison was based upon initial observations indicating that bumetanide was equipotent with furosemide at one-fortieth of its molar dosage.

(*c*) In two normal subjects the influence of 5 mg bumetanide upon the free water clearance was studied after control periods indicating a steady state of urine flow. One patient was studied after a water load of 1 500 ml orally followed by the hourly administration

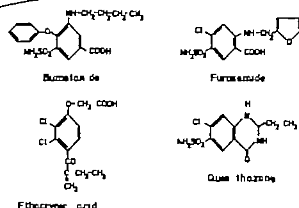


Fig. 1 Structural formulae of bumetanide and related diuretics.

of amounts equal to urinary output + 1 ml/min. The second patient was studied during hydropenia and antidiuresis induced by water deprivation for 15 hours and i.v. administration of pitressin in priming dose of 10 U and 10 U/hour in the sustaining infusion at 1 ml/min.

(*d*) In six patients the 4-hour effect of the combination of 1 mg bumetanide + 40 mg furosemide was compared to those of 2 mg bumetanide and of 80 mg furosemide using the rotation programme shown in Table I. A random allocation of patients to treatment schedules was secured. The objective was to examine whether the action of the combination of drugs fulfilled the criteria for simple dose addition effect. Dose addition describes combined effects of two drugs acting on the same receptors if doses of one drug are able to substitute for those of the other in proportion to their relative potency (15, 28, 30).

(*e*) In four patients the 4-hour effect of combinations of bumetanide with bendroflumethiazide, mercaptomerin, triamterene or spironolactone was examined. Single drugs and combinations were administered with intervals of one day.

(*f*) In five patients a preliminary attempt was made to estimate the maximally effective dose of bumetanide in

Table I. *Sequence of administration of diuretic treatments*

Treatments: A, bumetanide; B, placebo, C, furosemide

No. of pats.	Days 1	2	3
2	A	B	C
2	A	C	B
2	B	A	C
2	B	C	A
2	C	A	B
2	C	B	A

Table II. *Schedule for administration of bumetanide in trial f*

Pat. no.	Treatment days[a] 1	2	3	4	5
	Bumetanide dose (mg)				
1	2	6	4	8	10
2	4	8	10	6	2
3	6	10	8	2	4
4	8	4	2	10	6
5	10	2	6	4	8

[a] Interval of one day between treatment days.

patients with congestive heart failure using the rotation programme shown in Table II. A random allocation of patients to dosage schedules was secured. Different doses were administered with intervals of one day.

Long-term studies

Long-term effects and side-effects of bumetanide were observed in the following ways.

(*a*) In 13 patients the 24-hour urinary volume and electrolyte excretion were followed for 5 days after a control period of 2 or 3 days without treatment. Seven patients received 1 mg bumetanide, four 2 mg, and two 3 mg daily. The patients were given diet, added salt and potassium chloride supplement as described above.

(*b*) In 32 patients a long-term treatment was started during hospitalization and was later controlled in the out-patient clinic every month. After discharge the patients were recommended a full diet without added salt. Nine patients were started on 4 mg bumetanide/day, eight received 3 mg, and 15 2 mg daily. Twenty-four subjects were given 3 g potassium chloride/day while eight received 100 mg spironolactone daily.

Thirty-two patients were followed for 1 month, 23 for 2 months, 21 for 3 months, 19 for 4 months, 13 for 5 months, 10 for 6 months, 8 for 8 months, 4 for 11 months and 1 for 13 months. The total period of observation amounted to 148 months.

The following parameters were followed: serum sodium, chloride, potassium, standard bicarbonate, creatinine, protein, magnesium, Hb, leucocytes, differential counts, eosinophil leucocytes, thrombocytes, bilirubin, aminoaminotransferase and uric acid. Urine was examined for protein, glucose and abnormal cells.

(*c*) In 12 patients a comparison was made of the long-term effects of bumetanide and furosemide. After 2–3 months of treatment with bumetanide the patients were *treated for 1 month with furosemide given in an equivalent number of tablets*, whereafter they returned to the long term programme of bumetanide.

Biochemical methods

Sodium and potassium were measured by flame photometry (IL 143), chloride potentiometrically, standard bicarbonate by the Astrup microtechnique, magnesium by

atomic absorption spectrophotometry (IL 153), creatinine by modification of the Jaffé method, uric acid by an enzymatic method (uricase), bilirubin by modification of the method of Jendrassik et al., protein by biuret reaction, alanineaminotransferase by kinetic measurement with LKB automated photometer and net acid in urine (= ammonium ion + titrable acid—bicarbonate) according to the method of Jørgensen (17).

Serum and urine osmolality was determined by the freezing point depression method (Advanced osmometer). The free water clearance was calculated from the formula $C_{H_2O} = V - (U_{osm}/S_{osm})\ V$ where V = urine volume, U_{osm} and S_{osm} = osmolal concentrations of urine and serum, and where C_{H_2O} may have positive and negative values. Osmolal clearance is derived for the equation $C_{osm} = V - C_{H_2O}$ and indicates the amount of water required for excretion of urinary solutes if these were excreted in isotonic fluid (14, 16).

Statistical methods

Arithmetical means and standard errors of the means (S.E.M.) were calculated using conventional methods (33). In the comparisons of drug effects and of drug and placebo effects normal distribution could often not be assumed either for arithmetical values or after logarithmic transformation. Therefore the statistical analyses was performed by means of the Wilcoxon test for pair differences, which makes no assumptions with regard to distribution patterns (8).

Medication

Bumetanide was supplied by Leo, Ballerup, Denmark. The drug was delivered in tablets of 1 mg. The daily administration was as follows: dose of 1 mg was given at 9 a.m., dose of 2 mg as 1 mg at 9 a.m. and 1 mg at 1 p.m., dose of 3 mg as 2 mg at 9 a.m. and 1 mg at 1 p.m. Higher doses were divided in similar way. Furosemide (Lasix®) was used as commercially available tablets of 40 mg and administered in the same manner as bumetanide.

The potassium supplement used was Kaleorid® slow release potassium chloride tablet preparation which was tolerated very well (1). Potassium chloride supplements were used in this study to avoid severe potassium depletion and the associated risk of digitalis toxicity.

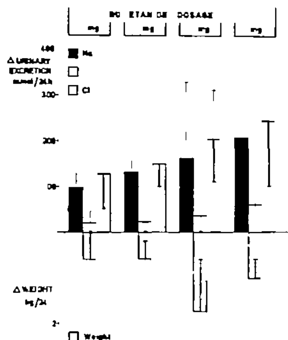


Fig. 2. Increments in electrolyte excretion and weight changes over 24 hours after bumetanide.

RESULTS

Short-term Effects of Bumetanide

Clinical effectiveness and individuality of responses

The natriuretic and diuretic responses to bumetanide in patients with congestive heart failure were impressive. Fig. 2 depicts increases in urinary excretion of sodium, potassium and chloride and weight losses induced over a 24-hour period by oral administration of bumetanide. The results are given as mean values and ranges for four groups of six patients who, according to estimated requirements, received 1, 2, 3 or 4 mg bumetanide, respectively.

The responsiveness of different patients to a given dosage of bumetanide varied considerably within each group and could frequently not be anticipated from the response to prior treatment. During diuresis sodium was the predominant cation accompanied by an increased potassium output. Chloride excretion was equal to the sum of sodium and potassium outputs (Fig. 2).

Comparison with furosemide

In order to evaluate the potency of bumetanide a comparison was made between the effect of this drug and the action of furosemide (3, 5, 19, 27, 34). Using the rotation scheme shown in Table I, four groups of 12 patients each were given both drugs and placebo tablets on alternating days.

As shown in Table III and Figs. 3 and 4 both drugs revealed significant effects in comparison with placebo tablets when evaluated in terms of natriuresis, kaliuresis, chloruresis, diuresis, osmolal clearance and weight loss. However, in the comparison between the two drugs

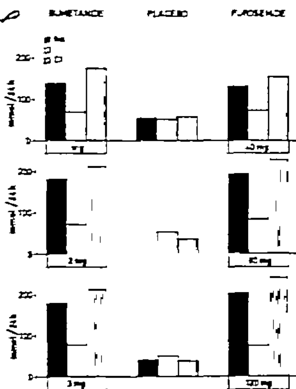


Fig. 3. Comparison of saluretic effects of bumetanide and furosemide.

no significant differences were found in any of the parameters mentioned.

It may therefore be concluded that bumetanide in the dose range 1 to 4 mg is equipotent with furosemide in the dose range from 40 to 160 mg.

Of particular interest is the finding that bumetanide in a dosage of 4 mg/day is able to induce a significant decrease of the negative free water clearance over 24 hours, i.e. the tubular reabsorption of solute-free water (Fig. 4).

Natriuretic response in special settings

Bumetanide proved to be an effective natriuretic agent in special settings like metabolic acidosis, hyponatraemia, hypochloraemia, hypokalaemia and decreased kidney function.

It is noteworthy that bumetanide is able to promote the same natriuresis, kaliuresis and diuresis in patients with metabolic alkalosis as in subjects with normal acid base status (Table IV).

Bumetanide was also found to be an effective diuretic in four patients with decreased renal function, i.e. creatinine clearances between 10 and 30 ml/min. In 44 patients with creatinine clearance values between 30 and 130 ml/min there was no correlation between clearance values and the 24-hour urinary sodium outputs ($r = 0.05$). Apparently the individuality of response to bumetanide mentioned above cannot be explained by variations in glomerular filtration rates.

Effects on electrolyte equilibrium and kidney function

Potassium. Bumetanide diuresis includes an increased urinary output of potassium. Largely this response is correlated with sodium output (Figs. 2–4 and Table III). As a result of the renal potassium loss there is a trend to development of hypokalaemia (Fig. 5 and Table III).

Chloride. The action of bumetanide involves an increase of chloride output, which usually exceeds the increment of sodium excretion (Fig. 2). The loss of chloride in excess of sodium in relation to extracellular ion levels accounts for the development of hypochloraemia (Table V and Fig. 5).

Acid base metabolism. As shown in Table V and Fig. 5 bumetanide diuresis induces a slight

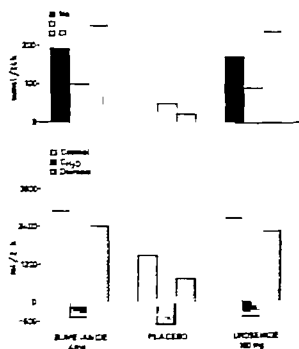


Fig. 4. Comparison of saluretic and diuretic effects of 4 mg bumetanide and of 160 mg furosemide.

Table III. *Statistical analysis of renal electrolyte water and solute excretion and of weight loss*

Units: sodium, potassium and chloride mmol/24 h, diuresis and osmolal clearance ml/24 h, b. wt. kg/24 h

	Mean 4-h values ± S.E.M.			Statistical significance of differences	
Renal output	Bumetanide (A)	Placebo (B)	Furosemide (C)	A–B	A–C
Trial 1	1 mg		40 mg		
Sodium	137.3±16.4	54.8±12.8	130.5±17.1		ns
Potassium	67.7±8.7	51.9±6.6	73.0±12.2	ns	ns
Chloride	174.0±22.3	57.8±14.1	153.8±18.1		ns
Diuresis	1 527±173	883±153	1 479±168		ns
Osmolal clearance	2 211±189	1 688±227	2 236±250	ns	ns
B. wt.	−0.48±0.17	+0.11±0.17	−0.43±0.14	ns	ns
Trial 2	2 mg		80 mg		
Sodium	180.8±32.3	30.8±12.9	194.3±47.9		ns
Potassium	71.4±8.4	52.5±6.1	80.8±8.5		ns
Chloride	211.1±29.5	37.3±12.5	229.3±35.9		ns
Diuresis	2 011±226	854±126	2 100±348		ns
Osmolal clearance	2 576±221	1 600±152	2 974±501		ns
B. wt.	−0.54±0.10	−0.25±0.05	−0.90±0.20		ns
Trial 3	3 mg		120 mg		
Sodium	179.8±29.4	40.8±11.4	202.3±38.1		ns
Potassium	77.3±6.7	48.7±7.3	74.1±7.2		ns
Chloride	212.8±32.6	41.1±14.8	241.9±42.9		ns
Diuresis	2 146±302	904±129	2 228±314		ns
Osmolal clearance	2 748±285	1 399±110	2 750±322		ns
B. wt.	−0.97±0.36	+0.33±0.14	−1.11±0.24		ns
Trial 4	4 mg		160 mg		
Sodium	193.1±21.1	18.8±7.5	174.4±28.4		ns
Potassium	98.8±7.9	48.7±3.4	92.9±6.6		ns
Chloride	254.4±22.6	23.5±9.4	241.8±36.1		ns
Diuresis	2 426±174	763±93	2 286±282		ns
Osmolal clearance	2 910±243	1 495±119	2 765±273		ns
Free water clearance	−484±103	−732±61	−479±95		ns
B. wt.	−1.06±0.10	+0.39±0.21	−0.89±0.35		ns

ns $p>0.05$, $p<0.05$, $p<0.01$, $p<0.001$

degree of metabolic alkalosis. In order to elucidate the mechanism involved in the trend to alkalosis the net acid excretion (= ammonium ion + titrable acid − bicarbonate) was measured in 18 patients. No significant difference was found between the mean values (±S.E.M.) after bumetanide (50±4 mmol/24 h) and after placebo (48±6 mmol/24 h).

Sodium. Bumetanide is able to induce a very significant natriuresis. As shown in Fig. 5 and Table V serum sodium and serum osmolality levels are maintained unaltered.

Kidney function. Serum creatinine concentration remained unchanged after bumetanide diuresis, indicating unaltered kidney function (Table V).

Mechanism and site of action of bumetanide

Usually bumetanide, when given orally has a rapid onset of action and induces a peak diuresis

Table IV *Diuretic response to bumetanide in relation to acid base status*

	Serum standard bicarbonate (mean ±S.E.M.)	
Parameters	<25.0 mmol/l	25.0 mmol/l
Na output (mmol/24 h)	164±18	152±21
K output (mmol/24 h)	78±5	76±6
Urinary volume (ml/24 h)	2018±175	2053±169
No. of pats.	26	22

Table VIII. *Comparison of long-term effects of bumetanide and of furosemide in 1 patients*

	Mean b.wt. ± S.E.M. (kg)	Mean difference in b.wt. ± S.E.M. (kg)	Statistical analysis
Bumetanide 1 month (before shift to furosemide)	67.27 ± 3.97		
		−0.48 ± 0.44	ns
Furosemide 1 month	67.75 ± 4.09		
		−0.03 ± 0.37	ns
Bumetanide 1 month (after shift from furosemide)	67.78 ± 4.16		

cantly during shift from one drug to the other. Similarly the mean values for serum electrolytes, creatinine, protein, uric acid and liver function tests remained unchanged.

DISCUSSION

Bumetanide given orally proved to be a very potent natriuretic and diuretic agent in patients with congestive heart failure. It was found to have an onset, peak and duration of action similar to that of furosemide (Fig. 6), but at approximately one-fortieth of the molar dosage. Therefore it seemed justified to compare the potency of these two drugs with each other and with a placebo in patients with heart failure. Since the effect of a diuretic varies with the pathophysiological status of the patient, the day of administration and the compound administered (18, 28), the patients were randomly allocated to the dosage schedule shown in Table I in order to reduce the variance. This procedure permitted a comparison of drug effects separate from the influence of other variables. The results, subjected to statistical analysis, showed that in 24-hour studies bumetanide in doses of 1, 2, 3 or 4 mg was equipotent with 40, 80, 120 or 160 mg furosemide, respectively with regard to urinary excretion of sodium, chloride, potassium, water osmolal clearance and weight loss.

Bumetanide was found to be a potent natriuretic in patients with metabolic alkalosis, metabolic acidosis, hyponatraemia, hypochloraemia and hypokalaemia. In terms of renal tubular action the effectiveness in the presence of metabolic alkalosis classes bumetanide with furosemide and ethacrynic acid and separates it from the mercurial diuretics which show a decreased effect in this setting (7, 27). Also in patients with decreased kidney function (creatinine clearance 10–30 ml/min) bumetanide appeared to be as effective as furosemide.

In short-term studies bumetanide promoted a significant rise in sodium and potassium excretion and an increase of chloride output, which was equal to the sum of sodium and potassium increments (Fig. 1). As a result of the disproportionate losses of potassium and chloride a trend to development of hypokalaemia and hypochloraemia was present, while serum sodium and serum osmolality levels remained unchanged. Since hydrogen ion excretion was unaffected, and since the increment of sodium output was excreted with chloride as anion, the most likely explanation of the development of metabolic alkalosis would appear to be a contraction of the extracellular volume with an unchanged total body bicarbonate content, i.e. a "contraction alkalosis" (6). Serum creatinine levels were unchanged during short-term bumetanide diuresis.

Bumetanide proved to decrease the positive free water clearance during water loading and to reduce the negative free water clearance, i.e. the tubular reabsorption of solute-free water during hydropenia and antidiuresis (Fig. 6). This double action separates bumetanide from the thiazide diuretics, which affect urinary dilution alone (10, 34, 36), and from the mercurial diuretics which have equivocal effects on both urinary dilution and urinary concentration abilities (14, 27). The capability of interfering with both urinary dilution and urinary concentration points to a locus of action of bumetanide in the ascending limb of the loop of Henle and classes this drug with furosemide and ethacrynic acid (14, 31, 35, 36). Actually the evidence presented in this study including the demonstration of a simple dose addition effect of the combination of bumetanide and furosemide, indicates that bumetanide is very similar to furosemide in terms of renal tubular action and suggests that bumetanide may act through the same renal tubular receptors. However, this preliminary conclusion does not rule out the existence of minor differences in renal tubular action between bumetanide and furose-

mide similar to those described between furosemide and ethacrynic acid (35).

Bumetanide can also be used in combination with other types of diuretics with beneficial results (Fig. 7). Additive natriuresis can be obtained in combinations with a thiazide and with mercaptomerin (3 16, 28, 30 34). A potassium-saving effect can be achieved in combinations with triamterene or spironolactone (20 23 29). This latter effect is in keeping with the interpretation that the increased potassium output during bumetanide diuresis is caused by an increased sodium-potassium exchange in the distal renal tubules due to an increased supply of sodium and to the activation of sodium-conserving homeostatic mechanisms (19 21 24 25 26). The fact that the increment of potassium output during bumetanide treatment is correlated with the rise of sodium output supports this hypothesis (32, 37).

In short-term studies bumetanide has been administered in doses up to 10 mg, and a preliminary study in patients with heart failure revealed a rising effect up to the level of 6 mg (Fig. 8). Further studies might modify this pattern. However for clinical purposes it should be pointed out that bumetanide is a very potent drug and that a high degree of individuality of response to bumetanide was found at any dose level (Fig. 1). Therefore it seems advisable to start the treatment with 1–2 mg, to watch the effect over the following 4–6 hours, and to adjust the next dose according to the initial response.

Bumetanide was also found to be very effective in long-term treatment of heart failure. When the patients were given potassium chloride or spironolactone, serum potassium levels were maintained and hypokalaemia occurred rarely While serum sodium levels remained normal, a trend to hypochloraemia and metabolic alkalosis was present. Apparently while the potassium supplement was adequate, the extra supply of chloride was insufficient. Serum magnesium level remained unchanged during long-term treatment with bumetanide. Apparently the increased output of magnesium described in acute furosemide and ethacrynic acid diuresis (9) has no bearing on long term treatment with this new drug.

The significant rise of serum protein after 3 months of treatment and the similar but statistically insignificant trend for Hb indicate that a slight degree of plasma volume contraction was maintained during long-term treatment. Similar degrees of plasma volume reduction have been reported during treatment with thiazides or furosemide in hypertensive patients (22) and in normal individuals (2) It is possible, but not proven that this response may play a role in the slight, but statistically significant rise in serum creatinine levels (Table VII) after 3 months of treatment (3). However it is also possible that the evidence for decreased kidney function may reflect the spontaneous course of disease in the patients studied. It is pertinent to point out that these patients maintained a normal serum sodium level and did not present evidence of marked overtreatment. Furthermore, the patients observed for more than 3 months did not show any additional rise in serum creatinine levels

Bumetanide was extremely well tolerated during the long-term treatment. Apart from muscle cramps in the calves in two patients symptoms or signs of intolerance of the drug were not seen. As already noted, leucocytes, eosinophil leucocytes and thrombocytes were unaffected. Liver function tests did not reveal significant changes.

A significant trend to a rise in serum uric acid was found after 3 months of treatment with bumetanide ($p < 0.01$). However no further increase was observed during the later course of bumetanide treatment, and gouty arthritis did not occur in this series. It is of interest that in 12 patients, where a comparison could be made, the mean value for serum uric acid remained at the same high level after 1 month of treatment with furosemide as found after bumetanide therapy Apparently bumetanide influences uric acid metabolism in a similar way to furosemide, ethacrynic acid and thiazides (3 4 19) A likely explanation of the hyperuricaemia is a decreased renal clearance of uric acid due to the sharing of a common secretory pathway with the diuretic. In the present material the trend to a decreased renal function during long-term treatment may be a contributory factor

It is well established that treatment with thiazides, furosemide and ethacrynic acid may provoke the manifestation of diabetes mellitus (19 38). This event did not occur in the present series, in which urinalysis for sugar remained negative in all patients. However more data are necessary before the influence of bumetanide

upon glucose metabolism can be precisely defined.

Also in long-term studies bumetanide proved to be equipotent with furosemide at one-fortieth of the molar dosage (Table VIII). It may be concluded, therefore, that bumetanide is one of the most potent diuretics available to-day and that this new drug on a weight basis is the most effective natriuretic agent developed so far. The synthesis of this highly effective compound sheds new light on the discussion of the relationship between chemical structure and natriuretic activity (12, 13). Fig. 1 depicts the chemical structures of the three "high ceiling" diuretics bumetanide, furosemide and ethacrynic acid. Apparently, since bumetanide has no halogen, the long held view that a halogen or a pseudohalogen, such as trifluoromethyl, as substituent in a neighbouring position to the sulfonamide group represents the "activating group" is no longer tenable. Similarly, the sulfonamide group itself cannot be of decisive importance for natriuretic activity since ethacrynic acid lacks this group. However, one common feature of all short-acting "high ceiling" diuretics is the presence of a carboxylic acid group ionised at physiological pH (1.). The significance of this group for the metabolism, renal excretion and renal tubular action of the drugs remains to be elucidated.

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HYPERVISCOSITY IN COLD ENVIRONMENT CAUSED BY A 6.5 S CRYOGLOBULIN IN A PATIENT WITH RHEUMATOID ARTHRITIS

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Abstract. A case of hyperviscosity in cold environment in a patient with rheumatoid arthritis and cryoglobulinaemia is reported. Ulcers and vascular disturbances (livedo reticularis) of the legs were possibly caused by a hyperviscosity syndrome in cold environment due to the presence of the 6.5 S cryoglobulin.

A unique group of M-proteins—found in cases of myelomatosis, Waldenström's macroglobulinemia, chronic infections, cirrhosis of the liver, rheumatoid arthritis—antigenically related to normal immunoglobulins possessing the distinctive property of temperature-dependent insolubility was described by Wintrobe and Buell (6) and termed cryoglobulins by Lerner and Watson (2).

We will describe a female patient with rheumatoid arthritis and cryoglobulinaemia showing a considerable increase of pain in the legs in a cold environment. The fact that in this case cryoglobulins were present which precipitated at temperatures lower than 24°, causing a hyperviscosity was in our opinion the reason for the pain in the lower legs in cold weather and the painful ulcers of the legs of this patient.

The pain in the legs was relieved when the temperature of the skin was elevated to normal.

CASE REPORT

A female, born April 25, 1917, had arthritic complaints and an ulnar deviation of the metacarpal joints. A livedo reticularis of the legs was diagnosed by a dermatologist. Treatment had consisted of butazolidin, aspirin, corticosteroids in low doses and injections for relieving intra-articular pain. Roentgenologically the deformities of hands and feet were typical of chronic arthritis. ESR was 65 mm/h. The Rose test was negative and antinuclear antibodies were absent. The LE phenomenon was negative. In the last 5 years of her life small ulcers appeared on her legs and especially on her feet, which were extremely painful in cold environment; in hands and arms no pain was felt. Local measures could temporarily heal the ulcers in the legs, but the pain in cold environment did not disappear. Skin temperatures were not measured. Histological examination of a skin biopsy of the legs showed wide capillaries in the cutis, and in the subcutis an artery with an obstructing thrombus was seen. Examination of the blood revealed the presence of a cryoglobulin. The results of the immunochemical investigations will be described further on.

About 18 months before her death a loud diastolic rumble on the right side of the sternum was heard for the first time. At that time the systolic BP was 180 and diastolic BP 65 mmHg. A diagnosis of aortic insufficiency was made. Repeated blood cultures were negative. In the last year before she died a progressive cardiac insufficiency was treated with digitalis, diuretics and salt restriction. The patient died in intractable cardiac failure.

Autopsy revealed a hypertrophy of the left and right ventricle of the heart, aortic insufficiency and endocarditis verrucosa, chronic congestion of the liver and spleen, arteriosclerosis of the kidneys and, to a lesser extent, of the other blood vessels. Unfortunately the ulcers of the legs have not been microscopied. Amyloid was not found.

Chemical investigations

Isolation and characterization of the cryoglobulin (1). The cryoglobulin was produced by the patient in the presence of a normal level of the major immunoglobulins: IgG 4.02, IgA 0.117 and IgM 0.110 g/100 ml plasma.

The amorphous gel-like mass, produced when the temperature of the gel was lowered to 4°C, was spun down at 1 000 rpm for 10 min at 4°C. The supernatant was removed. The protein content of the immunoglobulins in the supernatant was: IgG 1.54, IgA 0.117 and IgM 0.100 g/100 ml plasma as determined by the Mancini method (3) on Partigen immunodiffusion plates. The cryogel was dispersed in a large volume 0.15 molar NaCl and centrifuged at 4°C. The process of homogenizing and washing was repeated 5 times. The final precipitate, consisting only of IgG (γ, λ), IgG 1 and Gm (f+) molecules, as judged from immunoelectrophoresis, still showed the cryocharacteristics. So any involvement of IgM

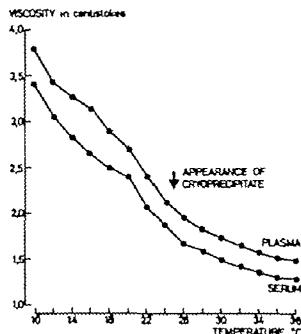


Fig 1 Relationship between viscosity and temperature for plasma and serum of the patient.

in the cryoprecipitation could be ruled out. The cryoglobulin sediment behaved as a single homogeneous component with S20 = 6.5 The amino acid composition and the carbohydrate content have been compared with normal IgG. Details are described elsewhere (1).

Viscosity measurement The measurements on the plasma and the serum were performed in an Ostwald viscosity meter (Ubbelohde tubes, volume 10 ml, P 31. Tamson, Holland) in a thermostated waterbath at different temperatures The viscosity was measured in the range of 10°–40°C and expressed in centistokes.

The relationship between viscosity and temperature is illustrated in Fig. 1 At temperature of about 24°C a white clouding of the plasma was observed. At this temperature the viscosity increased sharply This increase in viscosity at 24° was never seen in normals (Fig. 2) or in sera of patients with IgG and IgM elevation without cryoglobulinemia. A gradual increase in viscosity was seen in those cases.

DISCUSSION AND CONCLUSION

Probably the cryogel formation and the increase in viscosity are causatively related (4) The exacerbation of the pain in the legs in a cold environment could be caused, in our opinion, by the *hyperviscosity* at low temperatures. The skin temperature of resting lower legs in a cold environment may easily fall below 24 C. The relief of pain at higher temperature furthermore favours our assumption. The pain caused by low temperature, was only observed in the legs and not in the arms. Usually the inner temperature of the lower arms is higher than that of the lower legs, and the absence of painful ulcers in fingers or arms is therefore understandable. Furthermore the *livedo reticularis* was only visible on the legs. This phenomenon is caused by dilatation of the subpapillary venous plexus of the cutis. There are two venous systems in the cutis, a papillary and a subpapillary plexus. The first is provided with blood from an end artery and the second partly from the papillary plexus and partly from an arterio-venous shunt. In case of obstruction of an end artery the cutis is supplied with blood from the subpapillary plexus (5) In that case this plexus receives its blood supply only from the arterio-venous shunt functioning as a preferential channel in the capillary meshwork of the skin.

The hyperviscosity contributes to stagnation of the stream velocity of the erythrocytes in the capillaries and may also contribute to the livedo reticularis and possibly the ulcers. Cessation of blood flow in the superficial microvascular system for a period sufficiently long to cause ischaemic necrosis of the epidermis is rare but it may occur when there is a summation of several factors to increase the viscosity of the blood.

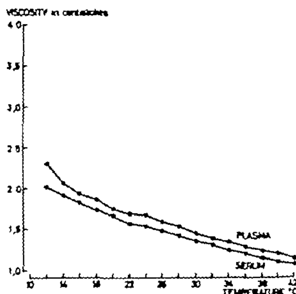


Fig 2 Relationship between viscosity and temperature for normal plasma and serum.

Low temperature predisposes to blood stasis, and this will be increased in the presence of a cryoglobulin. Since the amount of IgM was normal, and the cryoprecipitation involved only IgG molecules, the hyperviscosity was not caused by a macroglobulin. So, in our opinion, in this case a causal relationship between the pain in the legs and the presence of a 6.5 S cryoglobulin at temperatures below 24 C seems to be acceptable.

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BENIGN IDIOPATHIC PULMONARY HYPERTENSION?

Two Further Cases of Unusually Long Duration

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Abstract. Two further cases of unusually long duration of idiopathic pulmonary hypertension are reported and briefly discussed in relation to the pertinent literature.

In general, idiopathic pulmonary hypertension has a very poor prognosis. In a recent report of a patient who died 19 years after the disease started, Charters and de C. Baker (1) were unable to find a previous record in the literature of a duration from onset of symptoms until death longer than 18 years (4), while Nielsen and Fabricius (5) in a series of 14 patients recorded 3 who were still living after 15–20 years, and Wagenvoort and Wagenvoort in a recent pathoanatomical investigation of 110 cases of "vasoconstrictive primary pulmonary hypertension" (11) recorded 4 in whom symptoms had been present for over 20 years before death, with 29 years as a maximum. Usually the duration is much shorter averaging for instance 3.2 years in a material of 20 patients reported by Wood (12).

The purpose of the present communication is to report two further cases of unusually long survival in pulmonary hypertension of idiopathic type.

CASE REPORTS

Case 1

Female, born in 1935. Her family history is unremarkable. She was healthy as a child, but in 1944–45 she was not allowed to take part in physical training at school since it tired her unproportionately. In 1947 she had dyspnoea on moderate effort. From the time of menarche, in 1949, exertional dyspnea markedly increased and restricted the patient from climbing stairs. Frequent syncopal attacks occurred, but no edema, cardiac palpitations or anginous chest pains. Spontaneous, gradual improvement of the symptoms took place from the age of 16. The syncopal attacks disappeared and the patient was left with mild exertional dyspnea. She was able to take up employment as a waitress. She had repeated metrorrhagias, and underwent abrasio upon 5 occasions between the years 1950–55. In 1954 she had a spontaneous abortion. In the third month of gravidity increasing breathlessness and a few syncopal attacks again occurred but waned after the miscarriage.

In 1955 she appeared for a routine health examination. A heart lesion was suspected. She was admitted to the General Hospital in Malmö for further investigation in May of the same year. Physical heart findings included medium- to high-pitched systolic murmur with PM, grade II–III/VI in the 2nd–3rd left costal interspace near the sternum and widely split second heart sound with markedly accentuated pulmonic component. No incompensation signs were noted and there was no hepatomegaly. The systemic BP was 130/170 mmHg. Routine laboratory values were unremarkable. ECG showed right ventricular hypertrophy and somewhat accentuated and peaked P waves in lead V (Fig. 1 *a*). PCG verified auscultatory findings (Fig. 2 *a*). Chest X-ray revealed normal heart size and wide pulmonary arterial segment (Fig. 3 *a*). Right ventriculography demonstrated narrow peripheral pulmonary arteries and slow contrast passage through the pulmonary circulation (Fig. 4). No signs of intracardiac shunts were seen. Heart catheterization revealed moderate pulmonary hypertension (Table I) and no indication of intracardiac shunts in oxygen saturation analyses. Spirometry was normal.

The patient was discharged without medication and remained in a condition characterized by mild to moderate exertional dyspnea and easy fatigability for the ensuing years. She married, but had no further pregnancies. In 1960 she was reinvestigated at the Sahlgrenska Sjukhuset in Gothenburg. Renewed heart catheterizations were performed in 1960 and 1962 and showed essentially unchanged pulmonary arterial pressure levels (the patient has been tabularly represented as a case of primary pulmonary hypertension in a series reported by Wilhelmsen et al. (9 case XIII). Saluretics were instituted in 1960 and anticoagulants in 1962. Since 1965 bouts of endogenous depression have occurred, during which the patient has been treated with tricyclic drugs. In 1969 increased platelet adhesivity was recorded and salicylates were instituted. The patient's heart symp-

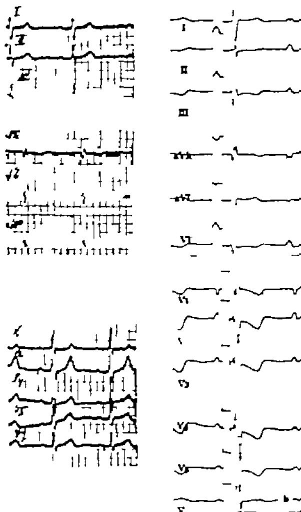


Fig. 1. ECG findings in case 1 in 1955 (*a*) and 1971 (*b*).

toms during this time remained largely stable. However, since 1960, slight intermittent ankle edema, occasional chest oppression and tachycardia on effort added to the other discomforts. Deterioration gradually started in 1968–69 with increasing exertional dyspnea, ankle edema, cardiac palpitations, tachycardia, fatigue, and recurring syncopal attacks. In Feb. 1971 digitalis and furosemide were added to her regimen.

For the last two years the patient has also noticed increasing whitening and numbness of the fingers and toes on cold exposure. No lasting skin changes have developed, however.

The patient was reinvestigated at the Medical Clinic in Malmö in Nov. 1971. Physical findings included slight clubbing of the fingers, peripheral cyanosis, mild orthopnea, and congestion of the neck veins with presystolic jugular venous pulsations. There was slight plethora. No other cutaneous changes were noted, and no edema or hepatomegaly. There was an evident right ventricular lift. Heart auscultation revealed widely split second heart sound with markedly accentuated pulmonic component, medium- to high-pitched diastolic murmur with PM, grade II–III/VI in the 2nd left costal interspace near the sternum and weak systolic murmur with PM parasternally in the 4th left costal interspace. BP was 105/80 mmHg. ECG indicated marked right ventricular and atrial hypertrophy (Fig. 1 *b*). PCG was consistent with severe pulmonary hypertension (Fig. 2 *b*). Chest X-ray exhibited predominantly right-sided cardiomegalia (heart volume 900 cm^3 corresponding to 600 cm^3/m^2 BSA), prominent pulmonary arterial segment and scant peripheral pulmonary vasculature (Fig. 3 *b*). Heart catheterization demonstrated increased pulmonary hypertension (Table I) and no indications of intracardiac shunts in the oxygen saturation analyses. Spirometry was unremarkable. Laboratory analyses revealed polyglobulia with serum Hb 19.5 g/100 ml and RBC 5.4 mill./mm^3. Serum bilirubin and liver enzymes were largely normal apart from serum γ-glutamyl transpeptidase 620 units. Plasma electrophoresis was unremarkable, as were extensive serological and organ antibody investigations. Detailed coagulation analyses were normal.

Case 2

Female, born in 1916. Her family history is unrevealing. During childhood she was healthy and routine examina-

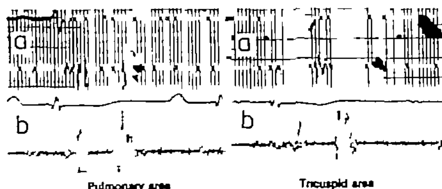


Fig. 2. PCG findings in case 1. In 1955 (*a*) accentuated second heart sound over the pulmonary area was recorded. In 1971 (*b*) accentuated and split second heart sound, and continuous, high-pitched diastolic murmur over the pulmonary area, presystolic murmur and short decrescendo murmur over the pulmonary and tricuspid areas were recorded.





Fig. 3. Frontal chest X-rays in case 1 in 1955 (*a*), and 1971 (*b*). Predominantly right-sided enlargement of the heart, wide pulmonary arterial segment, wide central pulmonary arteries, and scant peripheral pulmonary vasculature.

tions revealed nothing abnormal. During puberty however, the patient noted that she was not able to take part in physical activities as before, on account of exertional dyspnea. This was very slight at first, but at the end of puberty clearly limited her performance in running and other exercises which she had earlier enjoyed. She also had occasional tachycardia on effort and exertional, vertigo-like discomforts. She became accustomed to physically more restricted way of life and her symptoms remained essentially unchanged during the ensuing years and did not interfere with ordinary life. She married and had an uncomplicated parturition in 1941. From this time exertional dyspnea and tachycardia and attacks of "vertigo" again increased, and in 1942 she had syncopal attack. Syncopal attacks recurred with sparse intervals until 1960, from which time they have disappeared. The patient had second, complication-free delivery in 1947. Again the symptoms somewhat increased afterwards.

A routine chest X-ray in 1950 showed slight cardiomegalia (relative heart volume 585 cm^3/m^2 BSA) and markedly wide pulmonary arterial segment. In 1954 she sought medical advice. Physical examination revealed no edema, cyanosis or dyspnea at rest. A weak systolic murmur and markedly loud pulmonic sound were heard. A standard lead ECG revealed R/S quotient in lead I of 0.25/0.45 mV. A congenital cardiac lesion was suspected, but the patient refused further investigation and was discharged on hydrochloro-thiazide regimen.

From the end of the 50's slight ankle swelling occurred, and in 1960 the patient was admitted to the Medical Clinic in Malmö for cardiologic investigation. Physical findings included slight ankle edema, weak systolic murmur and markedly accentuated second heart sound in the 2nd–3rd left intercostal space near the sternum. BP was 120/75 mmHg. No cutaneous changes were noted. ECG was suggestive of moderate right ventricular hypertrophy and strain (Fig. 5*a*), and chest X-ray demonstrated predominantly right-sided enlargement of the heart (heart volume 750 ml, corresponding to 560 ml/



Fig. 4. Right ventriculography in case 1 in 1955. Wide central pulmonary arteries, and narrow peripheral pulmonary arteries with delayed contrast filling.

Table I. *Heart catheterization in cases 1 and 2*

a = wave, = v w e, M = mean, s = systolic, d = diastolic, id = initial diastolic, ed = end-diastolic, PCV = pulmonary capillary enous, — = data not obtained

		Pressures (mmHg)										
		Right atrium			Right entricle			Pulmonary artery				
Case no.	Month and year			M		id	ed	s	d	M	PCV M	Signs of intra-cardiac shunts
1	0555	6.5	4	2	49	0	0	46	13	28	8	No
1	1171	13	6.5	7	77	5	11	79	37	49	11	No
2	0460	12.5	10	9	56	5	7	57	26	39	—	No

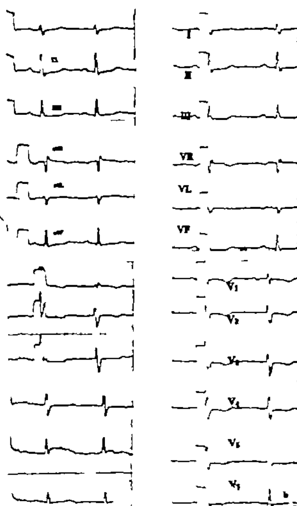


Fig. 5. ECG findings in case 2 in 1960 (*a*) and 1971 (*b*). Slight right frontal QRS axis deviation, increased frontal QRS-T angle, slight signs of right entricular hypertrophy in lead V and of right ventricular strain in leads V_1–V are seen in both ECGs.

m² BSA), wide pulmonary arterial segment and sparse peripheral asculature (Fig. 6). Angiocardiography showed the same findings and markedly slow contrast passage through the lungs (Fig. 7). There were no signs of intracardiac shunts. The PCG corresponded to auscultatory findings (Fig. 8 *a*). Heart catheterization revealed moderate pulmonary hypertension (Table I). The PCV pressure could not be obtained. Oxygen saturation analysis did not indicate intracardiac shunting. Routine laboratory values were unremarkable.

The patient cardiopulmonary condition has remained stable with exertional dyspnea restricting her from activities like climbing stairs, cleaning windows and ordinary walking for longer than few road blocks, tachycardia on effort, slight intermittent ankle edema, and occasional vertigo-like discomforts but no syncopal attacks. Digitalis has been added to her diuretic regimen. In 1967 she first noted that her fingers and later also toes whitened and felt numb on cold exposure. From this time a cutaneous depigmentation has gradually spread over the hands and feet but there have been no joint pains or restriction of the finger movements and no lasting skin ulcerations.

The patient was reexamined in Oct. 1971. Marked depigmentations were noted in the hands (Fig. 9) and feet, but there was no definite skin atrophy. The cutaneous changes were consistent with vitiligo. The patient refused skin biopsy. The radial pulses were normal, as was the skin temperature. Physical heart findings included right entricular lift, no definite enous congestion, and systolic murmur with PM, grade II/VI, in the 2nd–3rd left costal interspace near the sternum. BP as 120/80 mmHg. Slight lip cyanosis was recorded, but no edema or hepatomegaly. ECG revealed persisting signs of slight ventricular hypertrophy and strain (Fig. 5 *b*). Permission for recatheterization was not granted. Chest X-ray showed heart size 1 020 ml, corresponding to 560 ml/m² BSA, but the configuration was unchanged since 1960 (Fig. 6 *b*), as was the PCG (Fig. 8 *b*). Serum Hb was 15.7 g/100 ml and RBC 4.5 mill./mm³. Liver enzymes and other detailed laboratory analyses, including plasma electrophoresis and extensive serology were unremarkable. Coagulation analyses were normal. Serum B_{12} was 130 pg/ml (borderline alue). Organ antibody investigation revealed weakly positi thyroid cytoplasm and parietal cell reactions.





Fig. 6 Frontal chest X-rays in case 2 in 1960 (*a*) and 1971 (*b*). Predominantly right-sided cardiomegalia, wide pulmonary arterial segment, very wide central pulmonary arteries, sparse peripheral pulmonary vasculature.

DISCUSSION

As far as could be ascertained, congenital or acquired heart lesions, pulmonary parenchymatous disease or other extrinsic causes of pulmonary hypertension were not present in the two patients reported here. Both would seem to fulfil clinical criteria of "primary idiopathic or essential pulmonary hypertension" as elaborated by Wood (12). Their disease histories exhibit several similar features, including a plausible association with "factors related to female sexual maturity" commonly noted in essential pulmonary hypertension (8). The further course has been more progressive in case 1 however in whom deterioration has occurred after a relatively stable condition between the years 1955–68. In case 2 the course has remained remarkably benign. The main difference between her and case 1 seems to be that, after slight accentuation in connection with each of her two post partum periods, an apparent arrest of the progression of the disease has so far been lasting.

In the long run of the disorder both patients have developed discomforts of whitening and numbness of fingers and toes on cold exposure. Cyanotic, cold hands are common in primary pulmonary hypertension (12). However there is also the syndrome of "primary pulmonary hypertension and Raynaud's phenomenon" (11), which in many instances may represent clear-cut collagen diseases, such as disseminated lupus erythematosus (10) and, notably systemic sclerosis (7). Case 1 had no lasting skin changes, but in case 2 pronounced depigmentations of the hands and feet have developed. Her cutaneous changes are consistent with vitiligo rather than with true



Fig. 7 Right ventriculography in case 2 in 1960 (reflux of catheter into right atrium). Wide central pulmonary arteries, very narrow peripheral pulmonary arteries with markedly delayed and sparse contrast filling.

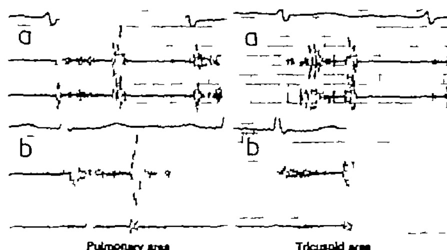


Fig. 8. PCG findings in case 2. In 1960 (*a*) accentuated and split second heart sound over the pulmonary area, presystolic and decrescendo systolic murmur over the tricuspid area. In 1971 (*b*) markedly accentuated second heart sound over the pulmonary area, continuous, high-pitched diastolic murmur over the pulmonary area, and medium- to high-pitched, decrescendo systolic murmur over the pulmonary and tricuspid areas.

Raynaud's disease. It must be noted, however that also vitiligo has been indicated to be related to autoimmune diseases (2).

Another feature which warrants brief consideration is the increased platelet adhesiveness recorded in case 1 in 1969. At a detailed coagulation in- m 1971 when the patient was main- upon a salicylate regimen, the platelet adhesiveness was normal and no other coagulation abnormalities could be verified. The enigma of increased thrombocyte aggregation also in cases of apparently non-thromboembolic, primary pulmonary hypertension has been thoroughly discussed by Oakley and Goodwin (6). The presence of "thrombosis-predisposing factors" in cases judged to be idiopathic pulmonary hypertension, together with the fact that microthromboembolic pulmonary hypertension and idiopathic pulmonary hypertension may share other similar clinical features, particularly in women in the



Fig. 9. Case 2. Areas of depigmentation in the hands.

child-bearing age (6) have supported conclusions that in this situation the two conditions represent modalities of closely related or identical disease processes (3). *However in chronic thrombo-embolic* pulmonary hypertension no survival longer than 20 years from onset of symptoms to death has been recorded (8).

The two patients described represent a disease duration of (25–) 27 and (35–) 40 years, respectively and would seem to be the longest surviving, clinically reported cases of idiopathic pulmonary hypertension hitherto. It is true that a morphologic diagnosis is lacking in both, but, according to Wood (12), "so far no case in which the final clinical diagnosis was primary pulmonary hypertension has been disproved by subsequent necropsy".

It is hoped that the present communication may further substantiate earlier conclusions (1) that rare cases clinically presenting as idiopathic pulmonary hypertension may exhibit a relatively stable and protracted course.

ACKNOWLEDGEMENTS

This study was supported by the Swedish National Association against Heart and Chest Diseases and the Hulda Ahlroth Foundation.

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BOOK REVIEW

Hypersensitivity to drugs. Edited by M. Samter and C. W. Parker. 439 pages. £11 — Sw. cr. 221.95. Pergamon Press, Oxford, England, 1972.

Nowadays it is a commonplace to say that our first question when confronted with an unusual disease picture should be: is it iatrogenic? The advent of large numbers of potent drugs has given pharmacotherapy something of a Janus head with two faces: the friendly side that cures many hitherto intractable diseases, the grim one that may cause suffering or even death. The present volume treats this important subject from many points of view. Duscombe, who writes about influences on the formal elements of the blood, has been able to treat his subject in the strict sense of the title. The "allergic" hypersensitivity has been well defined in hematology as well as in dermatology. The latter chapter was written by Schuppli in Basle. Both contain a wealth of information.

Another part of the volume contains chapters on drug-induced renal and hepatic disease. The group of nephrologists at Sahlgren's Hospital in Gothenburg have written a well-balanced study on nephrotoxic substances. The most important subject of phenacetin kidney is treated in detail and in a convincing way. Also the chapter on liver damage contains a very complete discussion. The last two subjects demonstrate very clearly that it is difficult to draw a line between "hypersensitivity" *sensu strictiori* and toxicity. On the other hand this fact poses the question whether the problem of pharmakogenetics, e.g. G 6 P D deficiency, should not have been given merely a passing mention. It is also evident that the work of Ishizaka and of the groups that have clarified the problem of allergic reagins should have been reviewed. The study of immunoglobulins type E have at last placed the problem of allergic reactivity on a firm biochemical basis.

The book should be used as an excellent source of knowledge in all big hospital libraries.

Jan G. Waldenström, Malmö

Congress Announcements

The Fifth International Diagnostic Course (diagnosis of the lung) will be held in Davos, Switzerland, April 5–11, 1973.

Sponsor: European Association of Radiology.

Main topics. Interstitial lung diseases and diseases of pulmonary vessels. Alveolar diseases, diseases of respiratory tract. Bronchography, tomography, needle biopsy, etc.

Instruction. One of the main purposes of the course 1973 is to familiarize the participants with the current thoughts of anglo-american pulmonary specialists. Up to now, the faculty includes a number of internationally known pulmonary experts.

Information. IDKD 5, Zurcher Hochgebirgsklinik, CH 7272 Clavadel-Davos, Switzerland or IDKD 5, P.O. Box 159, CH-8033 Zurich, Switzerland.

The Fourth International Symposium on Cardiac Pacing will be held in the Congress Centre Martinihal, Groningen, the Netherlands, April 17–19, 1973.

President: J. Nieveen, Groningen, the Netherlands.

Secretary General. H. J. Th. Thalen, Groningen, the Netherlands.

Secretariat. Symposium on Cardiac Pacing, Dept. of Cardiology, University Hospital, Groningen, the Netherlands.

The Fourth Congress of the International Society on Thrombosis and Haemostasis will be held in Vienna, Austria, June 19–22, 1973.

Main topics: 1) Biochemistry and biosynthesis of normal and abnormal clotting factors. 2) Clinical trials in the treatment of thromboembolic disorders. 3) Pathogenesis of thrombosis.

Symposia. 1) Blood-compatible biomaterials and thrombosis. 2) Haemorrheology and thrombosis. 3) Radioisotope techniques in blood coagulation and fibrinolysis. 4) Organ transplantation and coagulation. 5) Diagnostic methods in venous thrombosis. 6) Interaction between blood coagulation, fibrinolytic system and kinin system.

Deadline for registration of papers and submission of abstracts: Feb. 15, 1973.

All correspondence regarding the Congress: E. Deutsch, President, c/o Intercongress, Stadiongasse 6–8, A 1010 Vienna, Austria.

Acta med. scand. Vol. 193 pp. 145–146, 1973

EDITORIAL

SYSTEMATIC SERUM CALCIUM SCREENING—WILL IT BE NECESSARY?

Health control has become a medical catch-word, and screening of healthy populations with the aid of automatic analysers, ECG or other technical methods is becoming widely used. The value of such information regarding a healthy population has been discussed. Most doctors, however agree that a metabolic profile may be an important piece of information when starting a medical examination. The difficulty is inherent in the choice of parameters.

Some 30 years ago Fuller Albright of Boston, one of the most imaginative clinicians of our century made two important statements regarding the importance of hypercalcemia. 1) Hyperparathyroidism is not uncommon nor irreversible but may be very difficult to recognize clinically 2) Malignant tumours without bone metastases may possibly secrete a substance with hypercalcemic effects. He was right on both points (1).

This number of Acta medica scandinavica contains several papers on increase in serum calcium levels. One of the rare causes of this condition is hypoadrenocorticism. The problem is discussed in an interesting paper from Oslo. Another study presents results from the Uppsala group where for several years Ivar Werner has been the leading investigator in this field. The authors analyse and discuss the data from studies of calcium and phosphate excretion in patients with hyperparathyroidism. They find that in so-called essential hypercalciuria the possibility of normocalcemic parathyroid hyperfunction should always be strongly suspected. Neck exploration by an experienced surgeon is imperative in most cases.

Over the years Acta medica scandinavica has published a series of papers, e.g. by Bostrom and Wengle (2) and Irnell et al. (3) treating the diagnosis and results of therapy in hyperparathyroidism. Hellström of Stockholm was one of the early pioneers, and the tradition from the discoverer of the glands, Sandström of Uppsala (4), is obviously being kept alive in Scandinavia. The present number contains a study from a central hospital in Northern Sweden, presenting the preliminary results of systematic patient screening with automatic analysis of serum calcium levels. The population examined is very large and 100 persons might be diagnosed as hypercalcemic. Hyperparathyroidism was proven in 3 and regarded as probable in 11. No less than 11 patients suffered from malignant disease, but the largest group was taking thiazides.

It has always been difficult to establish the true incidence of a disease in an unselected population, and only large scale screening may give any hints. In the USA it has been stated by Boonstra that 50 patients with hyperparathyroidism were found in 10 years out of 50 000 patients (nearly all out-patients) who were consecutively screened with serum calcium determinations (1971).

The Swedish authors are inclined to regard this as being in accordance with their findings. Dr J F Dymling has informed me that the *annual* number of new cases of hyperparathyroidism in Malmö, where there is one hospital for a population of 250 000, has been 15 during a 3-year period. This would mean that 6/100 000 are found and treated per year—a minimum figure, but still high.

There is another aspect to the problem of unsuspected hypercalcemia, mentioned already in the introduction to this editorial, namely ectopic hormone production by cancer cells. Physicians with a wide clinical experience maintain that such

secondary hyperadrenocorticism from ectopic ACTH is more common than primary Cushing's disease. The same may well hold true, mutatis mutandis, for ectopic parathormone-like factors. The practical lesson is that search for a malignant tumour (pulmonary!) is always indicated—and *may be life-saving—if an elderly person has other*wise unexplained, marked hypercalcemia.

Perhaps the most important message that a medical journal may convey to its readers is ways of recognizing severe but curable disease. It seems to me that hypercalcemia with its often intricate differential diagnosis is one of the most urgent.

The question in the title of the editorial should evidently be answered in the affirmative.

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Jan G Waldenström

ERRATUM

In the *Program for Acta medica scandinavica for 1973* published in the first number of this year we regret that there has occurred a lapsus calami —if this expression may be used in the period of electric typewriters. It was stated that Birger Strandell has been in office as Editor for seven years. Most readers of the Acta must know that this is an understatement, as Strandell has been the Editor during twice that time or to be exact, 15 years. We regret this error

The Editors

Acta med. scand. Vol. 193, pp. 147–159 1973

QUANTITATIVE BACTERIAL CULTURE OF URINE

II. *Evaluation of 10 Different Screening Methods for the Diagnosis of Bacteriuria Compared with Results Obtained by the Dilution Technique*

René Vejlsgaard and Tage Justesen

From the Department of Clinical Microbiology, Institute of Medical Microbiology, Copenhagen, Denmark

Abstract. Starting off with the aim of finding an easily available, simple, reliable and inexpensive method for demonstrating significant bacteriuria in major health screening projects, representative standard bacteriological methods, transport agar methods and chemical methods have been studied in parallel on a total of 493 urine samples. The reference method employed was the dilution method in a flood-plate modification. The findings were as follows: 1) Methods conventionally employed (simple cultivation and phase-contrast microscopy) were too uncertain. 2) The chemical methods (Nitriatic® and Uriglox®) were undoubtedly specific, but too insensitive. 3) There was no essential difference between the four transport agar methods investigated (Uricult®, Inculator®, Urocult®, Calitube®). Both false positives and false negatives were at an acceptable level (< 3 %). These methods were found to satisfy the requirements of simplicity and reliability. The price per sample investigation can therefore be a decisive factor in the choice among screening projects, but not in the routine bacteriological laboratory. 4) In the routine bacteriological laboratory the loop method can be used with great advantage because of the high primary degree of differentiation, the calibration being constant within reasonable limits. However if this method is to be used, it requires trained and well instructed laboratory personnel, and there should be current control by the dilution method. 5) In future comparative investigations of screening methods the dilution method should be used as reference method.

Recognition of the possible sequelae in patients with bacteriuria, especially the immediate sequelae of bacteriuria in pregnancy, has led many investigators to initiate screening studies of major populations of various categories.

For this purpose the desirability of having an easy, simple, reliable and inexpensive method of demonstrating significant bacteriuria, especially one which could be used outside bacteriological laboratories, has been strongly emphasized.

In recent years a number of essentially different chemical methods have appeared on the market, for example the nitrite test in various modifications, the catalase test, the triphenyltetrazolium test (7, 10, 13) and finally the glucose oxidase test (9).

In addition, various transport agar methods have been marketed, based on modifications of either the dip-slide or the roll-tube principle (2, 4, 8). As far as the requirements mentioned above are concerned, these methods appear suitable, but they have neither been evaluated in a satisfactorily thorough manner nor against each other and only rarely with reference to conventional methods in the laboratories for routine clinical microbiology.

In particular, they have only been evaluated in a few cases in relation to the dilution method, which must be regarded so far as the most reliable procedure, although it is also the most time-consuming method for the quantitative evaluation of the bacterial content of urine.

The aim of the present investigation has been:

1) To compare a series of the methods mentioned above with quantitative urine cultivation by the dilution technique.

2) To examine whether it is possible to achieve rational advantages by the use of one or more of these methods in the microbiological laboratory of a medical department, without any loss of essential information.

3) To evaluate the methods mentioned with respect to their uncertainty in the daily routine, and particularly their use in practice without special bacteriological facilities being available.

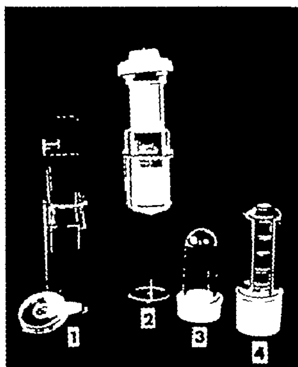


Fig. 1. The evaluated transport agar methods. (1) Uricult® (2) Inculator® (3) Urocult® (4) Cultube®

4) To evaluate the conventional bacteriological methods (simple cultivation and microscopy) from a quantitative point of view.

MATERIAL

During the period Sept. 1 1969 March 31 1970 a number of urine samples were selected daily and at random among the samples arriving at hospital laboratory for clinical microbiology.

Altogether 493 samples were collected either by means of the midstream technique (71 %), by catheter (19 %), or by the usual procedure of obtaining toilet urine (10 %).

All samples were first morning urine from hospitalized patients in surgical, medical and other special departments. The fasting period prior to the collection was varying, but presumably longer than 6 hours.

Of the total material, 28 % of the urine samples were collected from patients under chemotherapeutic treatment. All the clinically relevant micro-organisms were represented in the samples.

Of the samples examined 48 % came from men, 52 % from women. The mean age of the entire patient material was 49 years.

METHODS

Reference method

The dilution method (flood-plate technique) was the reference method selected for the comparative investigations. Successive logarithmic dilutions down to 10^{-4} were made of the thoroughly mixed urine in sterile saline. From each dilution 1 ml was immediately taken and 2 blood agar plates inoculated, 0.5 ml on each (11).

The plates were then incubated at 35°C for 18-24 hours. The colonies were counted on those plates where the count was estimated to be between 50 and 300 colonies per plate. The number of colonies is a mean of 2 counts and corrected to 1 ml urine.

In all cases a differential count was made, and the isolated colonies were subcultured and identified by means of the usual biochemical reactions.

Methods investigated

Altogether 10 bacteriological, chemical and conventional methods were studied.

I. Bacteriological methods

A. The loop method (streak plate)

The urine was inoculated onto both a blood agar and a lactose bromothymol blue plate by means of two calibrated loops of platinum-rhodium alloy (0.01 and 0.001 ml). (Supplied by A. H. Thomas Co., Philadelphia, USA, Inoculating Loop No. 7433-B and 7433-C.)

1) *Streak-plate I* refers to the loop-dilution method used in the routine bacteriological laboratory concurrently with the present investigation. By this method the result was given, after the conversion colony count/field × degree of dilution, as the highest number obtained for each bacterial species, the highest count being indicated by $> 10^5$ bacteria/ml urine.

2) *Streak plate II* was performed as part of the comparative investigation, along the same lines as indicated above. Furthermore, with this method, inoculation was performed on blood agar plates from ½ ml physiological saline with 0.001 ml loop, this amount of urine being first transferred to the saline by means of the same loop. In this way degree of dilution of urine of $10^3 \times 10^3$ 0.5 was obtained, which with this method provides means for closer quantitation of the bacterial content in urine with $> 10^5$ colonies/ml.

B. Dip-slide methods

3) *Uricult*® (supplied by Mecobenzon, Copenhagen) consists of microscope slide covered with nutrient agar on one surface and MacConkey' agar on the other. Each slide is supplied in sterile condition in small transparent plastic container with snap-on lid (Fig. 1). Inoculation was done by dipping the slide into urine so that both agar surfaces were completely covered, after which the slide was replaced in the plastic container and incubated at 22°C and 37°C.

Reading was done by examining the agar surfaces after removing the slide from the container. Both the reading of the number of species of micro-organisms and the quantitation of the total bacterial count were done for both types of agar by comparison with 5 standard pictures of agar surfaces, corresponding to the range 10^3–10^7 col./ml urine.

For comparison with the reference method the highest bacterial count read was used, converted to bacteria/ml urine.

4) *Incubator®* (supplied by Rœrad Products, Göteborg) consists of a flexible plastic "slide" which is divided up into countersunk fields (Fig. 1), in which various types of agar can be placed. The "slide" itself is fastened to the lid of a plastic container in which despatch and incubation can take place. Inoculation and reading took place on the same lines as for Uricult®.

Various bacteriological media in agar were dispensed in the fields mentioned, but as a basis for comparison in the present investigation only counts which were read-off either on Mueller-Hinton or on nutrient agar were used.

C. *Roll-tube methods*

5) *Urocult®* (supplied by Astra, Södertälje) consists of a transparent plastic sample tube, about 60 mm in length and 20 mm in diameter, furnished with a screw lid (Fig. 1). The inside of the sample tube is covered with an approximately 1 mm thick layer of transparent peptone-lactose-urea agar to which phenol red has been added. In addition, impregnated test strips are included for sensitivity determinations with respect to ampicillin, nalidixan acid, nitrofurantoin and sulfonamides. Inoculation was done by decanting urine into the sample tube until the agar was covered. After the urine was poured off, the lid was attached and the container incubated at both 22°C and 37°C.

Reading was done by examining the colonies through the agar layer counting the number of colonies within a limited area indicated by an accompanying template, and then by means of a standard table calculating the number of colonies/ml urine. The colour reaction of the agar was likewise noted.

In determining sensitivity by means of the strips impregnated with antibiotics the gradation "sensitive" was used where there was visible inhibition of growth, otherwise "resistant". As a reference method in these sensitivity determinations 20 mm discs were used with a prediffusion time of ½ hour.

6) *Cultube®* (supplied by Nunc, Roskilde). Like Urocult® this consists of a plastic sample tube, here 85 mm long and 30 mm in diameter. The inner surface is covered with agar medium with a red indicator which changes to yellow in the presence of bacterial growth. Cultube® has also windows (1 cm²) through which the count can be made. The amount of growth is read off and compared with an accompanying table, and the bacterial count is then given in col./ml urine.

II. *Chemical methods*

7) *The glucose reduction method* (*Uriglox®* supplied by Kabi through Bie & Berntsen, Copenhagen). The principle of this method is the demonstration that the small amounts of glucose found in urine from normal subjects are not present in urine containing bacteria, expressing the presence of glucose-fermenting bacteria.

A test strip was placed vertically and immersed approximately 1 cm in urine, with the impregnated part upwards, so that the strip became saturated in the course of a few minutes as a result of capillary action.

With the appearance of even a weak blue stain on the test part of the strip a negative result was recorded and, if the change in colour did not develop, a positive result. The strip was observed for at least 30 min if colour change did not occur.

8) *Nitrate reduction methods* (*Nitrituc®* and *Uritest®* supplied by Astra, Södertälje). The principle of this method is the demonstration of nitrite by change in colour indication with urine, demonstrating the presence of nitrate-reducing bacteria in the urine. In the form presented here the sample unit consists of an impregnated field, about 5 cm² in size, at the end of an elongated plastic holder, intended to be placed in the stream of urine while voiding.

In the present investigation the urine was poured over the test field from the collecting vessel and a negative reaction was read off as absence of colour change to reddish-violet within the course of 1 min.

III. *Conventional methods*

9) *Direct microscopy.* The investigation included phase contrast microscopy of the mixed, non-centrifuged urine at a magnification of 1 000 with the oil immersion objective. This microscopy recorded the main form of the bacteria as either cocci or rods, and the number per visual field was noted as follows: 1/10, 1/1, 10/1 and >10/1.

10) *Simple urine cultivation with semiquantitative evaluation.* The urine sample was centrifuged for 10 min (1 500 rpm) and then, dependent on a possible positive microscopy of a Gram-stained preparation, inoculated on to blood agar plates, which served at the same time for sensitivity determination. The plates were read 18–24 hours after incubation at 37°C on the following principle: "few" 2–3 col./streak, "some" 10–50 col./streak, "numerous" >50 col./streak.

Table I. *Influence of the technician's routine on the results obtained by the streak-plate method compared to the dilution technique*

Technician	No. of specimens	$>10^5$ col./ml flood-plate	False positiv streak plate $>10^5$	False negativ streak plate $<10^5$
A	50	13	0	0
B	50	18	1	0
C	53	18	0	0
D	52	11	2	6

RESULTS

I. *Bacteriological methods*

1) *Streak plate I versus dilution method.* Before the commencement of the investigation, studies were made on two factors as follows. *The variation in calibration over a lengthy period of use.* After daily use for 18 months, only insignificant differences were found in the volume transferred

Table II. *Paired tests of same specimens giving inconsistent results in routine streak-plate and serial dilution technique*

Organisms	Flood-plate col./ml urine	Streak-plate I 37°C, 24 h col./ml urine
		False pos. ~ 2.9%
E. coli	8.5 10^4	10^5
E. coli	3.0 10^4	10^5
Proteus mirabilis	5.8 10^4	10^5
Proteus morgani	3.0 10^4	$>10^5$
Staph. albus	7.0 10^4	$>10^5$
Staph. albus	1.1 10^4	$>10^5$
Diphtheroids	7.2 10^4	$>10^5$
Diphtheroids"	4.5 10^4	$>10^5$
Diphtheroids	5.8 10^4	$>10^5$
		False neg. ~ 12.7%
E. coli	4.0 10^5	10^4
E. coli	1.0 10^5	10^4
E. coli	3.4 10^5	10^4
E. coli	1.2 10^5	10^4
Klebsiella pneumoniae	1.7 10^7	10^4
Enterobacter aerogenes	1.5 10^7	10^4
Proteus mirabilis	4.7 10^5	10^4
Proteus mirabilis	3.0 10^5	10^4
Proteus mirabilis	9.8 10^7	10^4
Proteus vulgaris	3.2 10^5	10^5
Ps. aeruginosa	2.0 10^5	10^5
Strep. faecalis	6.0 10^5	10^4
Strep. faecalis	1.3 10^7	10^4
Enterococci	1.2 10^5	10^4
Staph. albus	1.8 10^5	10^4
Yeast	2.2 10^5	10^5
Yeast	5.2 10^5	10^4
Diphtheroids	2.8 10^5	—
Diphtheroids	7.1 10^5	10^4
Döderlein	4.4 10^5	10^4
"Döderlein	1.0 10^5	10^4
Döderlein	4.8 10^5	—
Döderlein	5.6 10^5	10^4

by the loop, determined by weighing. Next, *the skill of the individual laboratory assistant and its significance for the inoculation.* 4 laboratory assistants with varying experience in bacteriological work, from none (D) up to several years (C–A) daily inoculated 10 randomly chosen urine samples using calibrated loops. At the same time a trained technician carried out inoculation from the same samples by means of the dilution method as described. The results were then evaluated as false positive or false negative in relation to the result obtained by the dilution method, with 10^5 col./ml urine as the limiting value. They are shown in Table I, and it is seen that the use of calibrated loops requires a certain amount of experience and sustained training.

Table III. *Paired tests of same specimens giving inconsistent results in routine streak-plate with 1 step dilution and serial dilution technique*

Organisms	Flood-plate col./ml urine	Streak-plate II col./ml urine
		False pos. ~ 3.5%
E. coli	8.5×10^4	1.2×10^5
E. coli	8.5 10^4	1.0 10^5
Proteus mirabilis	9.8 10^4	2.0 10^5
Proteus morgani	3.8 10^4	5.0 10^5
Staph. albus	7.5 10^4	2.7 10^5
"Enterococci"	6.2 10^4	1.0 10^5
"Diphtheroids"	7.4 10^4	1.7 10^5
Diphtheroids"	5.9 10^4	1.8 10^5
"Diphtheroids	6.7 10^5	2.0 10^5
Diphtheroids"	1.9 10^5	5.0 10^5
		False neg. ~ 2.2%
Proteus mirabilis	6.3 10^5	1.0 10^4
Proteus morgani	1.2 10^5	9.7 10^4
Döderlein	1.2 10^5	2.4 10^4

Fig. 2 shows the results obtained with the calibrated loops in a routine bacteriological laboratory carrying out a large number of daily quantitative urine examinations and with several

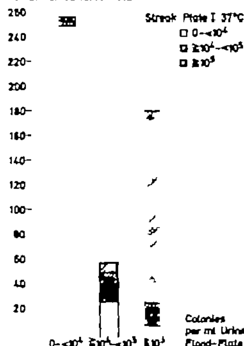


Fig. 2. Distribution of results obtained in the routine laboratory by streak-plate method compared to dilution method.

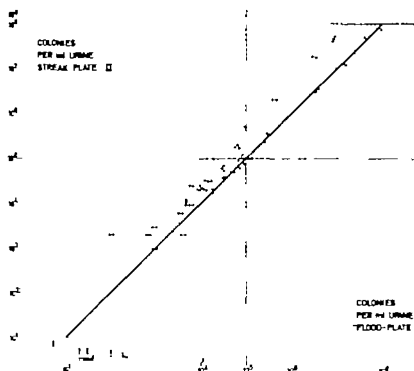
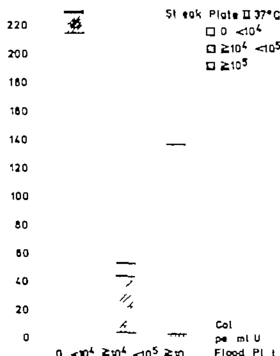


Fig. 3 Correlation between results obtained by streak plate with 1-step dilution and serial dilutions.



laboratory assistants with varying training. Evaluated in relation to the dilution method, with 10^5 col./ml urine as limiting value, there are found to be 2.9% false positives and 12.7% false negatives. Table II reviews the results of the paired tests for the inconsistent cases. An analysis of these results showed that the explanation of the large number of false negatives was the faulty evaluation of the streak-plate when the technician had not counted the colonies but merely estimated them too low and that it was a matter of isolated accumulations of false negatives, as they had a tendency to occur on days when the work was particularly pressing.

2) *Streak plate II versus dilution method.* Fig. 3 shows the graphic correlation between the two methods. As expected the streak-plate method gave slightly higher values than the dilution

Fig. 4 Distribution of results obtained by streak-plate method with 1-step dilution compared with dilution method.

Table II. *Paired tests of same specimens giving inconsistent results in routine streak-plate and serial dilution technique*

Organisms	Flood-plate col./ml urine	Streak-plate I 37°C, 24 h col./ml urine
		False pos. – 2.9 %
E. coli	8.5 10^4	10^5
E. coli	3.0 10^4	10^5
Proteus mirabilis	5.8 10^4	10^5
Proteus morgani	3.0 10^4	$>10^5$
Staph. albus	7.0 10^4	$>10^5$
Staph. albus	1.1 10^4	$>10^5$
Diphtheroids	7.2 10^4	$>10^5$
"Diphtheroids	4.5 10^4	$>10^5$
Diphtheroids	5.8 10^4	$>10^5$
		False neg. – 12.7 %
E. coli	4.0 10^5	10^4
E. coli	1.0 10^5	10^4
E. coli	*3.4* 10^5	10^4
E. coli	1.2 10^5	10^4
Klebsiella pneumoniae	1.7 10^7	10^4
Enterobacter aerogenes	1.5 10^7	10^4
Proteus mirabilis	4.7 10^5	10^4
Proteus mirabilis	3.0 10^5	10^4
Proteus mirabilis	9.8 10^5	10^4
Proteus vulgaris	3.2 10^5	10^5
Ps. aeruginosa	2.0 10^5	10^4
Strep. faecalis	6.0 10^5	10^4
Strep. faecalis	1.3 10^7	10^4
Enterococci	1.2 10^5	10^4
Staph. albus	1.8 10^5	10^4
Yeast	2.2 10^5	10^5
Yeast	5.2 10^5	10^4
Diphtheroids	2.8 10^5	—
Diphtheroids"	7.1 10^5	10^4
Döderlein	4.4 10^5	10^4
"Döderlein"	1.0 10^5	10^4
Döderlein	4.8 10^5	—
Döderlein	5.6 10^5	10^4

Table III. *Paired tests of same specimens giving inconsistent results in routine streak-plate with 1 step dilution and serial dilution technique*

Organisms	Flood-plate col./ml urine	Streak-plate II col./ml urine
		False pos. – 3.5 %
E. coli	8.5 10^4	1.2 10^5
E. coli	8.5 10^4	1.0 10^5
Proteus mirabilis	9.8 10^4	2.0 10^5
Proteus morgani	3.8 10^4	5.0 10^5
Staph. albus	7.5 10^4	2.7 10^5
"Enterococci	6.2 10^4	1.0 10^5
"Diphtheroids	7.4 × 10^4	1.7 × 10^5
Diphtheroids"	5.9 10^4	1.8 10^5
"Diphtheroids"	6.7 × 10^4	2.0 10^5
"Diphtheroids"	1.9 10^4	5.0 10^5
		False neg. – 2.2 %
Proteus mirabilis	6.3 10^5	1.0 10^4
Proteus morgani	1.2 10^5	9.7 × 10^4
"Döderlein	1.2 10^5	2.4 10^4

by the loop, determined by weighing. Next, *the skill of the individual laboratory assistant and its significance for the inoculation.* 4 laboratory assistants with varying experience in bacteriological work, from none (D) up to several years (C–A) daily inoculated 10 randomly chosen urine samples using calibrated loops. At the same time a trained technician carried out inoculation from the same samples by means of the dilution method as described. The results were then evaluated as false positive or false negative in relation to the result obtained by the dilution method, with 10^5 col./ml urine as the limiting value. They are shown in Table I, and it is seen that the use of calibrated loops requires a certain amount of experience and sustained training.

Fig. 2 shows the results obtained with the calibrated loops in a routine bacteriological laboratory carrying out a large number of daily quantitative urine examinations and with several

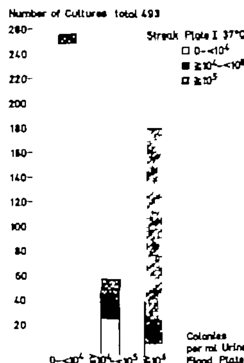


Fig. 2. Distribution of results obtained in the routine laboratory by streak-plate method compared to dilution method.

to be easily handled, although it gives a little trouble in the form of a sharp edge on the microscope slide. There are only insignificant difficulties in reading the results, which should be done outside the transport tube. Species differentiation is difficult at $> 10^3$ col./ml urine. The keeping power evaluated by the shrinkage of the medium and the degree of contamination, was good at +4 C. It was easy to dispatch, although the thickness of the transport tube was too slight for safety in the post. This method demonstrated 65% of the species found by the dilution method.

4) *Inculator® versus dilution method.* This method, which does not differ in principle from Uricult® gave similar results with respect to false positives and false negatives—although in a smaller material. Inculator® occupies somewhat more space than Uricult® without giving any greater reading surface· its keeping power was the same as that of Uricult® and it was easy to dispatch.

5) *Urocult® versus dilution method.* The results obtained with Urocult® are shown in Figs. 7 and 8 compared to the dilution method. The paired

Table IV *Paired tests of same specimens giving inconsistent results by Uricult® at 22°C and serial dilution technique*

Organisms	Flood-plate col./ml urine	Uricult 22°C col./ml urine 24 h	48 h
		False pos.	
		3%	3 ₄
E. coli	3.4 10^4	10^6	10^6
Klebsiella pneumoniae	3.2 10^4	10^6	10^6
Proteus mirabilis	3.9 10^4	10^6	10^6
Strep. faecalis	4.8 10^4	10^6	10^6
"Diphtheroids	4.5 10^4	10^6	10^6
		False neg.	
		19%	18
E. coli	3.0 10^5	0	0
E. coli	1.7 10^5	0	—
Enterobacter aerogenes	1.5 10^5	0	0
Staph. aureus	1.4 10^5	0	●
Staph. aureus	1.0 10^6	0	—
Staph. aureus	9.0 10^5	0	—
Staph. albus	5.0 10^5	0	—
Yeast	1.6 10^5	0	0
"Diphtheroids"	2.8 10^5	0	0
"Diphtheroids	5.8 10^5	●	—
Döderlein	5.6 10^5	0	0

Table V *Paired tests of same specimens giving inconsistent results by Uricult® at 37°C and serial dilution technique*

Organisms	Flood-plate col./ml urine	Uricult 37°C, 24 h col./ml urine
		False pos. = 3.4
E. coli	8.0 10^4	10^6
E. coli	8.5 10^4	10^6
E. coli	3.0 10^4	10^6
E. coli	3.4 10^4	10^6
Klebsiella pneumoniae	3.2 10^4	10^6
Enterobacter cloacae	2.0 10^4	10^5
Proteus mirabilis	5.8 10^4	10^6
Proteus mirabilis	3.9 10^4	10^6
Proteus morgani	3.0 10^4	10^6
Strep. faecalis	4.8 10^4	10^6
Enterococci	4.0 10^4	10^6
Staph. aureus	6.5 10^4	10^6
Staph. albus	1.8 10^4	10^6
Staph. albus	1.9 10^4	10^6
"Diphtheroids	4.5 10^4	10^6
"Diphtheroids	5.8 10^4	10^6
"Döderlein	3.0 10^4	10^6
		False neg. = 3.0
Ps. aeruginosa	9.8 10^5	10^4
Staph. aureus	2.0 10^6	10^4
Staph. aureus	1.4 10^5	0
Staph. aureus	1.0 10^6	10^4
Staph. albus	1.8 10^6	10^4
Staph. albus	2.0 10^6	10^3
Yeast	6.0 10^6	10^4
"Döderlein"	2.0 10^7	10^4
"Döderlein	4.4 10^6	10^4

tests showing inconsistent results are given in Tables VI and VII, at 22 C and 37 C, respectively.

There are a considerable number of false negative results on incubation at room temperature. The false negative results with the large number of tubes showing no growth at this temperature might suggest that both the amount of substrate and its composition were insufficient, so that this method, too cannot be recommended for use at 22 C.

The results obtained after 24 hours at 37 C showed good agreement and are presumably in the region of the scatter of the methods, but both the false positives and the false negatives lie in the critical regions, where renewed examination is recommended.

The Urocult® method has likewise been tested with respect to colour change, thus giving a rough qualitative evaluation of the bacte-

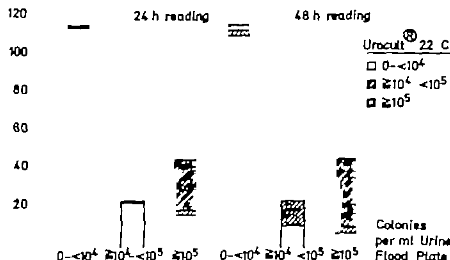


Fig. 7. Distribution of results obtained by Urocult® at 22°C after 24 h and 48 h incubation compared to the dilution method.

species. In the case of *E. coli* and *Proteus* spp. the reaction appears to have been satisfactory; the results are shown in Table VIII.

The set of strips supplied with the Urocult® for determining the sensitivity of the bacteria found has been examined and the results are shown in Table IX. The number of bacteria which were "false sensitive" to the chemotherapeutics most commonly employed in urinary tract infections was small.

As a general conclusion it may be said that Urocult® was easy and rapid to work with, but the results were slightly difficult to read. It was not so convenient with respect to differentiation of species and the subsequent isolation, but the possibility it gave for a rough differentiation between bacteria, and determination of their sensitivity reduced its negative aspects. The keeping power of the material was good and it was convenient for postage. The method demonstrated 56% of the species found in the dilution method.

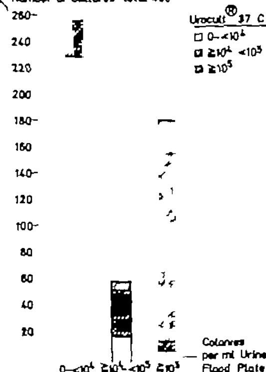


Fig. 8. Distribution of results obtained by Urocult® at 37°C compared to the dilution method.

6) *Cultube® versus dilution method.* Like Urocult® the method is based on the roll-tube principle and gave no results which differed from those obtained with Urocult®. Cultube® does not allow any differentiation apart from growth or non-growth, it cannot be used for sensitivity determinations. It was difficult to get the layer of agar to remain attached to the tube. The tubes examined had certain disadvantages, connected with the manufacturing procedure, which can easily be corrected. The keeping power was as indicated, and the material was easy to dispatch by post.

II. Chemical methods

7) *Uriglox®.* The glucose content was examined by this method in 329 urine samples. Fig. 9 shows

Table VI. *Paired tests of same specimens giving inconsistent results by Urocult® at 22°C and serial dilution technique*

Organisms	Flood-plate col./ml urine	Urocult® 22°C col./ml urine 24 h	48 h
		False pos.	
		0 %	2 %
E. coli	8.0 10^4	—	10^5
E. coli	3.1 10^4	—	10^5
Enterococci	2.2 10^4	—	10^5
		False neg.	
		37 %	16 %
E. coli	3.0 10^5	0	—
E. coli	5.3 10^5	0	—
Klebsiella pneumoniae	1 7 10^7	0	0
Klebsiella oxytoca	5.7 10^5	0	—
Proteus mirabilis	1.1 10^5	10^4	10^4
Ps. aeruginosa	2.2 10^5	0	—
Enterococci	1 4 10^5	0	—
Enterococci	5.4 10^5	0	—
Enterococci	1.0 10^5	0	—
Staph. aureus	1.4 10^5	0	0
Staph. albus	1.0 10^5	0	—
Staph. albus	5.0 10^5	0	0
"Diphtheroids"	7.1 10^5	10^4	10^4
"Diphtheroids"	1.4 10^5	0	10^4
Yeast	1 6 10^5	0	0
"Döderlein	1.0 10^5	0	—

Table VII. *Paired tests of same specimens giving inconsistent results by Urocult® at 37°C and serial dilution technique*

Organisms	Flood-plate col./ml urine	Urocult® 37°C, 24 h col./ml urine
		False pos. – 2.2 %
E. coli	8.0 10^4	> 10^5
E. coli	8.5 10^4	> 10^5
E. coli	3.2 10^4	10^5
Enterobacter cloacae	2.0 10^4	10^5
Strep. faecalis	4.8 10^4	10^5
Enterococci	1.6 10^4	10^5
Staph. aureus	6.5 10^4	10^5
		False neg – 6.1 %
E. coli	1.2 10^5	10^4
Proteus mirabilis	1.1 10^5	5 10^4
Proteus vulgaris	3.2 10^5	5 10^4
Ps. aeruginosa	2.1 10^5	10^4
Staph. aureus	2.0 10^5	10^4
Staph. aureus	1.0 10^5	< 10^4
Staph. aureus	1 4 10^5	0
Staph. albus	1 4 10^5	< 10^4
Staph. albus	1.8 10^5	10^4
"Diphtheroids	7.1 10^5	5 10^4
Döderlein	4.4 10^5	< 10^4

the results, a positive reaction signifying no presence of glucose in urine thus $> 10^5$ bacteria col./ml urine, compared with the results obtained by the flood-plate method. Out of 225 urine samples with $< 10^5$ col./ml urine 5 false positive Uriglox® reactions were found (2 %). Out of 104 urine samples with $\geq 10^5$ col./ml urine 55 were found to be false negative (53 %).

If these results are evaluated more closely omitting patients under treatment and only considering samples collected by catheter or midstream urine, and among these only considering samples with a content of bacteria which could ferment glucose in vitro, there remain 18 samples (35 %) which must be recorded as false negative.

The method must be regarded as not quite so simple to carry out with regard to screening or possible self-examination.

8) *Nitritic®* (*synonym Urnitest®*). Fig. 10 shows the results obtained by this method for the entire material. Calculation gave 1 4 % false positives and 47 % false negatives. A closer evaluation as described for Uriglox® also gave 47 % false negatives. *If the non-consistent samples are* analysed, no explanation is found for the false positives. Among the false negatives there were a great number of bacteria which reduced nitrate in vitro. The method was easy to carry out and could, if necessary be administered by the patient himself

III Conventional methods

9) *Phase-contrast microscopy* This traditional method of the bacteriological laboratories is still

Table VIII. *Colour reactions observed in Urocult® media in relation to isolated bacterial species*

Bacterial species grown	Yellow	Neutral	Red
E. coli	44	1	0
Enterobacter spp.	1	2	0
Klebsiella spp.	1	1	2
Ps. aeruginosa	0	3	0
Proteus spp.	0	1	9
"Diphtheroids"	0	1	1
Staphylococci spp.	0	10	3
Enterococci	4	2	1

Table IX. *Correlation between sensitivity tests obtained with sensistrips in Urocult® and disc diffusion technique*

Sensitivity pattern	Sulfonamide	Ampicillin	Nalidixic acid	Nitrofurantoin
Correlation	70	80	70	84
False resistant	23	13	26	12
False sensitive"	7	7	4	4
Total	100	100	100	100

a very popular screening method. The results obtained with this technique by one and the same investigator have been compared with respect to sensitivity and specificity with those obtained by quantitative cultivation. Fig. 11 shows how frequently even trained investigators found bacteria by microscopy without any organisms being present. A number of the false positive results may have been bacteria but present in the urine simultaneously with the presence of antibiotics.

If the material is grouped according to whether cultivation has shown only cocci or rods, and these findings are compared with the findings by phase-contrast microscopy then the results are as shown in Fig. 12 *a* and *b*. This shows how difficult it can be to differentiate cocci from other formed elements in the urine.

Microscopy thus appears to give relatively

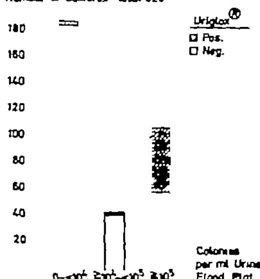


Fig. 9. Distribution of results obtained by Uriglox® compared to the dilution method.

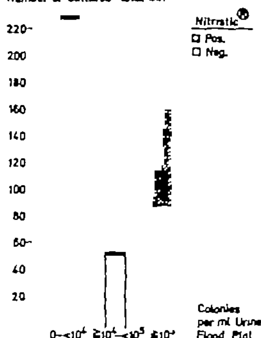


Fig. 10. Distribution of results obtained by Nitrastic® compared to the dilution method.

many false positive results, but also a number of false negative results, even when done by trained laboratory assistants. The method requires some routine, but is rapid if the necessary microscopic equipment is available.

10) *Simple cultivation.* Table X shows the semi-

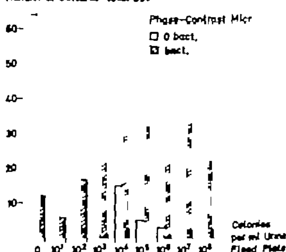


Fig. 11. Distribution of results observed by phase-contrast microscopy (bacteria or no bacteria seen) compared to the dilution method.

Table IX. *Correlation between sensitivity tests obtained with sensistrips in Urocult® and disc diffusion technique*

Sensitivity pattern	Sulfonamide	Ampicillin	Nalidixic acid	Nitrofurantoin
Correlation	70	80	70	84
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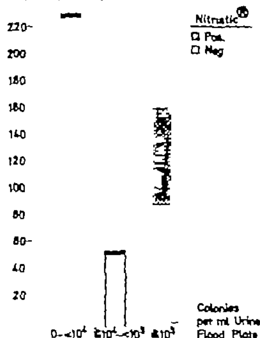


Fig. 10. Distribution of results obtained by Nitratic® compared to the dilution method.

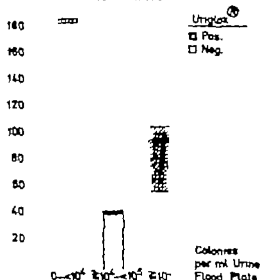


Fig. 9. Distribution of results obtained by Uriglox® compared to the dilution method.

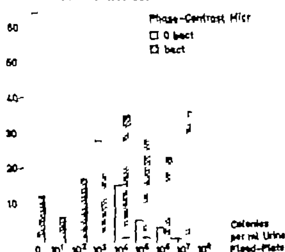


Fig. 11. Distribution of results observed by phase-contrast microscopy (bacteria or no bacteria seen) compared to the dilution method.

Table IX. *Correlation between sensitivity tests obtained with sensistrips in Urocult® and disc diffusion technique*

Sensitivity pattern	Sulfonamide	Ampicillin	Nalidixic acid	Nitrofurantoin
Correlation	70	80	70	84
False resistant	23	13	26	12
False sensitiv	7	7	4	4
Total	100	100	100	100

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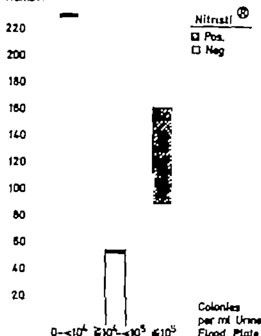


Fig. 10. Distribution of results obtained by Nitrur® compared to the dilution method.

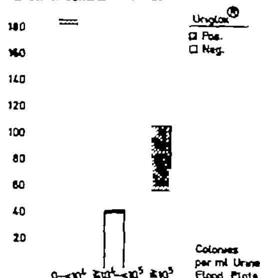


Fig. 9. Distribution of results obtained by Uriglox® compared to the dilution method.

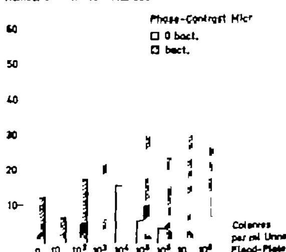


Fig. 11. Distribution of results observed by phase-contrast microscopy (bacteria or no bacteria seen) compared to the dilution method.

Table IX. *Correlation between sensitivity tests obtained with sensistrips in Uricult® and disc diffusion technique*

Sensitivity pattern	Sulfonamide	Ampicillin	Nalidixic acid	Nitrofurantoin
Correlation	70	80	70	84
False resistant	23	13	26	12
False sensitive	7	7	4	4
Total	100	100	100	100

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If the material is grouped according to whether cultivation has shown only cocci or rods, and these findings are compared with the findings by phase-contrast microscopy then the results are as shown in Fig. 12 *a* and *b*. This shows how difficult it can be to differentiate cocci from other formed elements in the urine.

Microscopy thus appears to give relatively many false positive results, but also a number of *false negative results*, even when done by trained laboratory assistants. The method requires some routine but is rapid if the necessary microscopic equipment is available.

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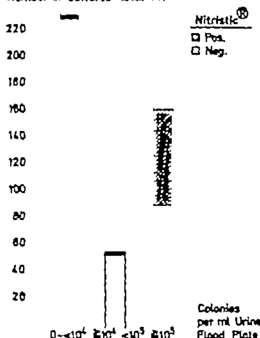


Fig. 10. Distribution of results obtained by Nitristic® compared to the dilution method.

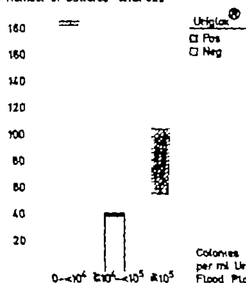


Fig. 9. Distribution of results obtained by Uriglox® compared to the dilution method.

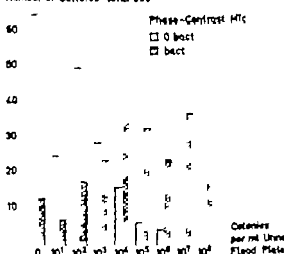


Fig. 11. Distribution of results observed by phase-contrast microscopy (bacteria or no bacteria seen) compared to the dilution method.

Table IX. *Correlation between sensitivity tests obtained with sensistrips in Uricult® and disc diffusion technique*

Sensitivity pattern	Sulfonamide	Ampicillin	Nalidixic acid	Nitrofurantoin
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Total	100	100	100	100

a very popular screening method. The results obtained with this technique by one and the same investigator have been compared with respect to sensitivity and specificity with those obtained by quantitative cultivation. Fig. 11 shows how frequently even trained investigators found bacteria by microscopy without any organisms being present. A number of the false positive results may have been bacteria but present in the urine simultaneously with the presence of antibiotics.

If the material is grouped according to whether cultivation has shown only cocci or rods, and these findings are compared with the findings by phase-contrast microscopy then the results are as shown in Fig. 12 *a* and *b*. This shows how difficult it can be to differentiate cocci from other elements in the urine.

Microscopy thus appears to give relatively

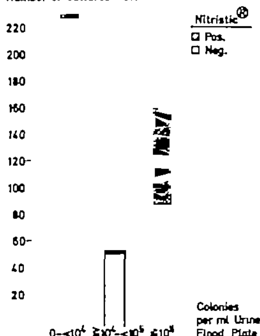


Fig. 10. Distribution of results obtained by Nitristic® compared to the dilution method.

many false positive results, but also a number of false negative results, even when done by trained laboratory assistants. The method requires some routine, but is rapid if the necessary microscopic equipment is available.

10) *Simple cultivation.* Table X shows the semi-

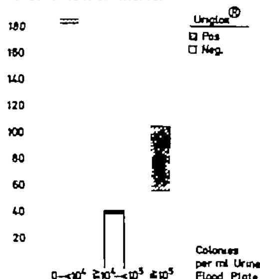


Fig. 9. Distribution of results obtained by Uriglox® compared to the dilution method.

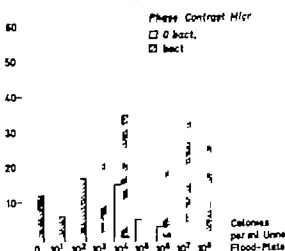


Fig. 11. Distribution of results observed by phase-contrast microscopy (bacteria or no bacteria seen) compared to the dilution method.

sured diagnosis of urinary infection. Nor does the present investigation provide a completely sufficient basis for this, although the laboratory report of a semi-quantitative estimate may be a good guide provided that the collection of urine and its transport have been carried out according to instructions. Nevertheless it must be admitted that the designation a few may leave many in doubt, and this category in particular emphasizes the necessity for quantitation. It has been claimed that phase-contrast microscopy could be of value when carried out on uncentrifuged or possibly centrifuged urine. The present study has shown that, even for the trained investigator this may be a misleading investigation.

Over the years the need for a quantitative evaluation of the bacterial content of urine has led to the expenditure of much time and money on the search for rapid, simple reliable and easily available methods for diagnosing significant bacteriuria.

The results achieved with these methods have varied considerably and have not always been comparable. The reasons are to be sought in the difference between one patient material and another the type of urine sample the method and time of collection, and the evaluation of the results of the investigation, in this connection particularly the reference method chosen.

The reference method chosen in the present in gation has been the dilution method, in the flood-plate modification, because it more easily permitted inoculation onto several plates. Although this method involves the possibility of sources of error it must be emphasized that it is the most reliable method, and comparative investigations of various methods have also shown it to be the most suitable for quantitative bacterial counts. It follows that this method should also be employed in epidemiological investigations for research purposes. However the method requires time and material and cannot therefore so easily be employed in major screening studies.

Many methods have been marketed for this purpose, in most cases investigated separately or together with poorly chosen reference methods.

In the present study selected and more recent methods have been investigated, particularly those which appear to satisfy the requirements of simplicity reliability and economy

In our hands the chemical methods Nitristic® and Uriglox® have not given convincingly good results, even though the manufacturers instructions have been followed as closely as possible. They were found to be of *high specificity* (few false positives) but of *low sensitivity* (many false negatives). In the case of the nitrite reaction this corresponds to the findings of many other investigators (7 10 13) whereas the results with respect to Uriglox® are contradictory Scherstén et al. (9) for example, find good agreement. Our many false negatives tell against its use A further disadvantage of these methods is the rigorous demands made on the patient in the form of fasting and retention of urine in the bladder for about 6 hours before micturition. This in itself means that, in spite of considerable efforts and many investigations, the ideal chemical screening method has so far not been found.

The publication by Mackey and Sandys (8) of the transport agar method introduced new means for the diagnosis of urinary infections.

By working in the field" it is possible to avoid the inconvenient refrigerated transport in the dispatch of the inoculated sample tube to a bacteriological laboratory and it may even be possible immediately to sort out the negative samples in advance.

The significance of such transport has been demonstrated by several investigators (3).

In the present investigation four different modifications of the transport agar method were evaluated. The results obtained do not differ considerably and are good enough to recommend their clinical use. The few false positive results would presumably have been revealed in consecutive investigations, but the difficulties of distinguishing between 10^4 and 10^5 col./ml urine emphasize that samples which lie in this region should be checked. The restricted number of false negatives must be due to the differences in the substrates employed. The results show that incubation at 22 C, as recommended by some investigators (1) should not be employed. In addition to being reliable and simple the methods were also rapid. In the clinical bacteriological laboratory it was relatively easy to distinguish between various species at values $< 10^5$ col./ml. With respect to transport, storage, and keeping power the various methods were equivalent. It may be added that the investigations carried out here were done by trained technicians, so that,

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wherever they are likely to be put into operation, the methods should be tried out by personnel not used to the procedure (e.g. nurses, midwives, secretaries).

Which of the methods are to be preferred in screening investigations must remain a question of price per investigation. The price of the methods examined here varies considerably and the requirements stipulated by the postal authorities for the dispatch of infectious material were not fully satisfied by all.

The aim of this study was also to evaluate the possible use of the transport agar method in routine bacteriological laboratories. The methods appear practicable but owing to the fact that better substrates were already being used as a routine, more easily permitting species differentiation and determination of sensitivity in one and the same sitting, the transport agar media can only be recommended for transport and screening with reference to elimination of negative samples, while they may possibly be employed in less well equipped laboratories with limited bacteriological expertise. If the principle followed is to have the inoculated transport agar sent to the laboratory then the reply will be delayed by 24 hours, since the laboratory must first isolate the organism from the transport agar and make a pure culture. Transport of urine samples in refrigerated containers must still be recommended when possible.

In the routine bacteriological laboratories the use of the streak-plate method can be recommended, but only if a number of requirements are satisfied. First, the personnel must be used to the routine and fully aware that the number of bacteria must not be guessed at but counted. The high number of false negative samples found in these investigations may presumably be ascribed to this cause. It is strongly recommended that a simultaneous check should be made by parallel culture of random samples, using both the streak plate method and the dilution technique.

With regard to the streak-plate method, it has been claimed that it should not be used as a routine because of the variation of the calibration of the loop with time (6). Our investigations, like others' (5), show that, in spite of frequent sterilization of the loop by flaming, the calibration remains rather constant.

Having thrown some light on the aims set out in the Introduction, the conclusion is that we must recommend continued use of the dilution methods (flood-plate, pour-plate) in scientific screening investigations, and particularly in the evaluation of new screening methods.

The method is too difficult and time-consuming for routine use in bacteriological laboratories, where we must recommend the use of the streak plate method, once routine has been achieved and control introduced.

In major health screening projects, where it is desired to screen for the incidence of urinary tract infection and the cost of dispatch per sample must decide the choice of method, the use of transport agar methods can be recommended for the time being. This recommendation presupposes that the transport agar medium is incubated and read "in the field" and that only the positive and doubtfully positive specimens are dispatched to the bacteriological laboratory for further investigation. If such reading cannot take place, then, taking everything else into consideration, transport in refrigerated containers must be recommended.

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Acta med. scand. Vol. 193 pp. 161–165 1973

SERUM PHOSPHATE AND CALCIUM AND PHOSPHATE EXCRETION AT DIFFERENT LEVELS OF SERUM CALCIUM IN HYPERPARATHYROIDISM

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Abstract. The excretion of phosphate and calcium and the level of serum phosphate have been compared and related to different levels of serum calcium in 73 cases of verified hyperparathyroidism (HPT). Variations in the dietary regimen over short periods had little influence on the results. The phosphate parameters were fairly closely related to the degree of hypercalcemia. Patients with pronounced hypercalcemia had more hypercalciuria than those with moderate elevations, but pronounced hypercalciuria was also found in the group of normocalcemic HPT. These patients are easily mistaken for essential hypercalciuria.

Until specific hormone assays are available for extensive routine use the diagnosis of hyperparathyroidism (HPT) still essentially rests on the clinical history and rather simple determinations of calcium and phosphate in serum and urine. Significantly elevated serum calcium (Ca/s) values are not always obtained in cases with subsequently confirmed HPT. Sometimes it is difficult to decide whether other diseases might be responsible for the hypercalcemia when present.

Three out of 4 patients with HPT have hypercalciuria (10, 16). Low serum phosphate (P/s) is usually found in 50% of the cases and a high excretion, expressed as a reduced tubular reabsorption of phosphate (TRP) elevated phosphate clearance (C_P) or phosphate excretion index, is found in 2 out of 3 patients (9). (The denotation TRP is used here and in the following, as it is a conventional expression for the renal handling of phosphate. No stand has been taken on the question whether a small or large fraction of urinary phosphate has in fact been tubularly secreted.) The biochemical findings mentioned, particularly hypercalciuria, often occur without HPT being the cause. Although the diagnostic value of these laboratory investigations is limited, the results obtained in a case of suspected HPT may always be considered more or less in agreement with the diagnosis.

The aim of the present study was to describe the biochemical pattern most commonly found in HPT at different Ca/s levels.

MATERIAL

The material consists of the patients investigated in similar scheme during the period 1960–68. Seventy-three subjects, 25 males and 48 females, most of the latter postmenopausal, were subsequently found to have HPT verified by successful surgical exploration. The distribution of the Ca/s values is shown in Fig. 1 together with definitions of the patient groups and subgroups in respect to Ca/s levels. Twenty-five patients had Ca/s values below 5.5 mEq/l (group I), 5 of whom must be considered normocalcemic (group I a). Ca/s levels within the range 5.5–6.0 mEq/l were found in 21 cases (group II) and above 6.0 mEq/l (group III) in 17 cases. Ten subjects with elevated serum creatinine levels were equally distributed between groups II and III. Levels of 2.5 and 2.0 mg/100 ml were found in one patient each. In two cases each the serum creatinine was 1.5 and 1.8 mg/100 ml. Four patients had 1.4 mg/100 ml.

METHODS

During varied dietary conditions determinations were made of Ca/s, P/s, 24-hour calcium excretion, TRP and C_P. An initial examination on normal diet was followed by a period of high calcium (1 200 mg/day) and high phosphate intake and another period of low calcium (< 200 mg/day) and low phosphate intake with oral aluminium hydroxide. On the 5th–6th day on the special diets TRP and C_P were estimated between 8.00 and 10.00 a.m. Often, but not always, the

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A preliminary report was presented to the Swedish Societies for Endocrinology and Nephrology Nov 1969

Table I. *Mean values and S.D of Ca/s, P/s, 24-hour Ca excretion TRP and C_P at different Ca/s levels and on different dietary regimens in patients with HPT*

Groups refer to definitions presented in Fig. 1
0 = normal diet, + = diet rich in calcium and phosphate, – = calcium-poor diet and aluminium hydroxide

Diet	Group I		Group II		Group III	n	Group I		Group III b	n	Normal values	
Serum calcium (mEq/l)												
0	5.48±0.30	25	5.65±0.21	31	6.63±0.73	17	4.88±0.12	5	7.88±0.35	4	4.70±0.25	40
+	5.31±0.20	22	5.70±0.22	23	6.71±0.60	11	5.26±0.16	5	7.50±0.22	3	–	
–	5.36±0.26	24	5.74±0.20	29	6.76±0.63	16	5.02±0.13	5	7.55±0.46	4	–	
Serum phosphate (mmol/l)												
0	0.94±0.18	25	0.87±0.15	31	0.81±0.22	17	0.90±0.13	5	0.70±0.16	4	1.10±0.17	40
+	0.95±0.17	22	0.87±0.16	23	0.80±0.20	11	0.90±0.18	5	0.83±0.18	3	–	
–	0.89±0.17	25	0.78±0.14	29	0.77±0.24	16	0.86±0.19	5	0.68±0.10	4	–	
Urinary calcium excretion (mEq/24 h)												
0	13.5±6.0	24	11.9±4.7	29	14.3±6.4	16	18.4±7.8	5	15.0±4.6	4	5.63±2.2	40
+	15.8±5.7	22	14.9±7.2	21	18.2±6.3	10	18.0±6.6	5	17.3±5.7	3	–	
–	14.2±5.0	24	12.3±5.9	28	19.1±9.2	15	17.2±4.4	5	18.0±4.1	3	–	
Tubular reabsorption of phosphate (%)												
0	86.2±6.2	23	81.0±10.5	26	72.7±12.3	11	84.5±5.6	4	76.5±1.6	2	90.5±2.3	40
+	84.4±6.8	22	82.8±9.1	21	71.6±10.5	11	83.2±6.9	5	63.5±2.6	2	–	
–	88.7±4.4	25	83.7±10.1	26	74.6±9.0	15	89.2±3.3	5	70.8±4.4	4	–	
Clearance of phosphate (ml/min)												
0	13.3±3.2	22	14.1±6.7	26	18.0±3.0	11	15.8±7.7	4	16.0±1.0	2	11.0±2.9	40
–	16.6±7.2	22	18.1±6.0	20	20.3±4.7	11	15.4±6.1	5	25.5±1.6	2	–	
–	12.2±4.2	25	14.0±7.0	26	19.2±8.2	15	14.4±3.8	4	25.0±3.1	4	–	

patients were investigated on all three dietary regimens. Only routine facilities were available for the supervision. Calcium (11) and creatinine determinations (12) were usually performed with an Autoanalyzer. Organic phosphate was determined by manual technique (3). TRP was calculated as $(1 - C_P/C_{cr})\ 100$.

RESULTS

Mean values, the standard deviation and the number of investigated subjects on the different dietary regimens are shown for Ca/s, P/s, urinary calcium 24 h, TRP and C_P in Table I. Figures are given for the three main patient groups as well as for the small subgroups with the lowest (I *a*) and highest (III *b*) calcium values. The normal values refer to 40 patients on normal diet and without renal or metabolic disease.

The different dietary regimens had a limited influence on the results. As expected, the highest calcium levels and the lowest P/s values were obtained on phosphate deprivation. Only in group I *a*, 5 subjects, was Ca/s somewhat higher when a calcium-rich diet was given. The excretion of calcium, too, was usually not strikingly influenced by the diet, although a few cases responded by an increased output after phosphate deprivation. With low supply of phosphate and calcium slightly lower C_P and higher TRP values were found. By setting a lower normal limit of 85% for TRP on normal diet, 87% on phosphate deprivation and 83% on a rich supply of calcium and phosphate, the diet no longer had an influence on the frequency of abnormal values obtained. After this operation low values, "positive for HPT" were found in approx. 30% of cases in group I, 50% in group II and 80% in group

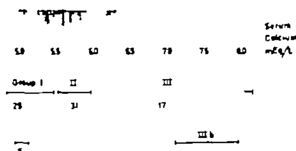


Fig. 1. Distribution of Ca/s values (mean of 3–6 determinations) in 73 subjects with HPT and division of the material into 3 main groups and subgroups.

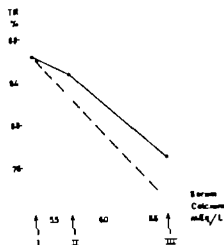


Fig. 2. TRP at different Ca/ levels in HPT. The mean values are shown before (-) and after (——) the exclusion of subjects with raised serum creatinine level.

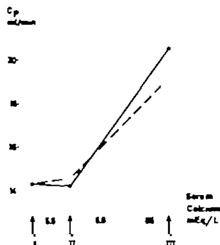


Fig. 3. C_P at different Ca/ levels in HPT before (●- -●) and after (O- -O) exclusion of the subjects with elevated serum creatinine level.

III. Probably because of sometimes inaccurate urine collections C_P was less useful than TRP. On a phosphate-rich diet the P/s was 0.7 mmol/l or less in 18% of cases in group I, 21% in group II and 45% in group III.

The glomerular filtration rate is known to be important for the renal handling of calcium and phosphate. Therefore the 10 subjects with an elevated serum creatinine were excluded from the material before evaluation of the excretion at different levels of Ca/s. For TRP and C_P the mean values in groups I–III are shown before and after this correction for renal impairment. The mean of the values observed on different regimens was used, and it is demonstrated in Figs. 2 and 3 that Ca/s and the excretion of phosphate were highly related to each other.

In Fig. 4 the calcium excretion in the different groups on the 3 dietary regimens is presented. Group I is divided into groups I *a* and I *b*. The lowest figures of calcium excretion were observed in group II. A considerably higher excretion was found not only in the individuals with advanced hypercalcemia but also in the normocalcemic cases.

DISCUSSION

Phosphate excretion parameters and P/s are known to be unreliable as diagnostic criteria for HPT. A few additional comments may be made from the viewpoint of the present study. The biochemical pattern is more likely to include a low P/s and a low TRP the more pronounced the hypercalcemia. To some extent this may explain why their use, suggested from observations in patients with clearcut HPT, has not been found of much value for the differential diagnosis in less obvious cases. However, even in borderline cases



Fig. 4. Mean daily excretion of calcium in HPT at different Ca levels. O = normal diet, + rich supply of Ca and P, – – = low calcium diet and phosphate depletion.

of HPT P/s or TRP values in the upper half of the normal range might be considered as evidence against HPT as they do not fit into the usual biochemical profile. The similar results found on different dietary regimens suggest that it is usually unnecessary to prolong the hospital stay for the purpose of establishing a controlled intake of phosphate and calcium. Obviously more clearcut effects of dietary regimens are likely to be obtained during studies for longer periods under metabolic ward conditions. This would not be a possible routine procedure, as a reasonable suspicion of HPT is so commonly encountered. The relatively short dietary periods in the present study may partly explain why it was not possible to verify the findings of Reiss and Alexander (8) that most cases of HPT obtain a low TRP on a regimen rich in phosphate and calcium. Neither was it possible, in almost all cases, to observe significant hypercalciuria on phosphate deprivation as found by Pronove and Bartter (7)

The U shaped relationship between Ca/s values and urinary calcium excretion might at first glance appear surprising (Fig. 4) If the tubular reabsorption of calcium is increased in HPT (6 13) an almost normal excretion should be expected when hypercalcemia is not present. However regardless of the etiology hypercalciuria may precipitate kidney stones and the cases with values of Ca/s within the normal range had all been surgically explored because of considerable problems from renal calculi. The patients in group I a may thus be a small selected part of a large number of mainly asymptomatic subjects with normocalcemic HPT Many of the patients with hypercalcemia had never suffered from kidney stones A considerable increase of the number of verified borderline cases in our clinic has followed upon a more active attitude towards cases with only moderate symptoms.

It is not possible to prove however that normocalcemic hypercalciuria in HPT is due solely to the selection of patients with kidney stones. Other mechanisms might be considered, as little is known of the normal pathophysiological development of HPT It has been suggested that the effect of parathormone on the kidney can be expressed as elevating the threshold for calcium excretion (5) This threshold is probably depressed by the antagonistic hormone calcitonin (4) and in the presence of a raised production of this hormone the serum calcium level might remain normal in HPT As there is good evidence that calcitonin is not antagonistic to the parathormone effect on the gut, hypercalciuria might still follow because of an increased gastrointestinal absorption of calcium. A high secretion of the two main hormones regulating Ca/s would theoretically also be a possible explanation of many cases of idiopathic hypercalciuria. In this disorder Coe et al. have reported that the parthyroid hormone level is higher than normal, a possible explanation of which would be an unknown constant stimulus to parathormone secretion (2). A parathyroid adenoma developing in such a case would thus in fact be tertiary"

The present material contained 5 subjects with hypercalciuria and Ca/s values in the upper normal range. Four of these were postmenopausal women similar to the cases described by Yendt and Gagne (16). Some male patients with hypercalciuria and normocalcemic HPT have been reported by Adams et al. (1). In most other published cases of normocalcemic HPT the urinary calcium excretion has been excessive too (15). In other materials (1) as in our experience, such patients often have P/s and TRP within the lower normal range. It is important not to accept these patients passively as essential hypercalciuria as a surgical neck exploration might be curative.

Hypercalciuria and kidney stone disease in our cases disappeared after the removal of parathyroid adenomas. The risk of finding normal conditions at the surgical exploration might be reduced by the use of calcium infusion tests for evaluation of these patients (14) and in the future possibly by the determination of parathormone and calcitonin in serum. In spite of the difficulty in recognizing these cases we would like to stress the point that hypercalciuria in borderline hypercalcemia or together with normal Ca/s values, is sometimes an important sign of HPT

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Acta med. scand. Vol. 193, pp. 167–173 1973

CLINICAL FINDINGS IN PATIENTS WITH HYPERCALCAEMIA

A Preliminary Investigation Based on Biochemical Screening

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Abstract. With multi-element chemical testing 90 patients with hypercalcaemia (serum calcium 5.6 mEq/l or more) have been found during 9 months. The frequency of hypercalcaemia was roughly estimated at 1–2‰/year. According to clinical data the patients were divided into five main groups: 1) verified primary hyperparathyroidism, 2) probable primary hyperparathyroidism, 3) possible primary hyperparathyroidism, 4) hypercalcaemia of other known causes, and 5) hypercalcaemia of unknown cause. *The first three groups comprise 20% of the total number* of patients and the last two 40%. The frequency of hyperparathyroidism seemed to correspond to that found in other studies. Drugs of different kinds, especially thiazides, were the most common cause of hypercalcaemia, closely followed by hyperparathyroidism and malignant diseases. A few case reports are described.

In 1969 the biggest multichannel instrument operating today, the AutoChemist, constructed by G and I Jungner was installed in the Laboratory of Clinical Chemistry at the 800-bed County Hospital in Östersund, Sweden, where it has been in operation since Jan. 1970.

*The laboratory serves, besides the County Hos*pital, another 1 200 hospital beds, most of which are situated in Östersund. The outpatient departments, district physicians and private practitioners, who are also served by the laboratory receive 300 000 patient visits per year. In 1969 before the AutoChemist was operating, the number of tests performed by the laboratory was 600 000 including haematology and urinary tests. The number of analyses in 1970 increased to 1.1 mill.

At the time of this investigation 20 analyses were performed on each serum sample (Table I). The principle of multi-element chemical testing has in our case caused a tenfold increase in the number of calcium analyses performed. Thus probable evaluation of the true incidence of hypercalcaemia may be obtained.

The accuracy and precision are controlled by running primary and secondary standards before and in between patient samples. For calcium analyses the accuracy is within 0.1 mEq/l and the coefficient of variation has not been allowed to exceed 2%.

THE SURVEY

From Sept. 1970 to May 1971 all patients with serum calcium values above 5.5 mEq/l have been recorded. During these 9 months 107 patients with at least one serum calcium value above 5.5 mEq/l were found. In 17 cases clinical data have not been available. The following *presentation therefore deals with only 90 cases of hyper*calcaemia. Distribution curves in this article are based on the highest recorded serum calcium value in each patient.

The population in the county of Jämtland is nearly 125 000. Since we have found 90 cases of hypercalcaemia during 9 months, the incidence of hypercalcaemia in this survey is 100/year 100 000 inhabitants. Many patients have of course not been investigated and we therefore believe that the true incidence of hypercalcaemia may be 200/year/100 000 inhabitants.

With regard to laboratory data and data obtained from patient records the patients have been divided into five main groups (Table II).

1. Patients with PAD-verified adenoma of the parathyroid glands have been classified as verified primary hyperparathyroidism cases. Three patients with primary hyperparathyroidism during 9 months is less than the normal 8–10/year.

2. The second group, probable primary hyperparathyroidism, contains patients with several high calcium and low phosphorus values. Most of these patients are awaiting operation and this may explain why the number of verified cases is less than normal during the period of this investigation. In one case the patient refused further investigation and operation.

3. The third group, possible primary hyperparathyroid-

Table I. *The AutoChemist test battery*

Analysis	Methodological principle	Measuring range
Creatinine	Jaffe without deproteinization	0–30 mg %
Total protein	Biuret (Weichselbaum 1946)	0–12 G „
Albumin	Bromcresol green (Rodkey 1965)	0–5 G %
Sodium	Flame photometry (Eppendorf)	100–180 mEq/l
Potassium	Flame photometry (Eppendorf)	2–10 mEq/l
Chloride	Hg $(SCN)_2$ (Zall et al. 1956)	60–140 mEq/l
Calcium	Flame photometry (Eppendorf)	2.0–9.0 mEq/l
Bilirubin	Diazo reaction (Jendrassik, Grof)	0–25 mg %
Thymol	Maclagan 1944 (Shank Hoagland units)	0–35 U
Alk. phosphatase	4-amino-antipyrine (Roos 1963)	0–40 IU
GOT	Reitman–Frankel 1957 (Karmen units)	0–250 U
GPT	Reitman–Frankel 1957 (Karmen units)	0–150 U
LDH	Babson–Phillips	0–1 800 U
Serum iron	Direct nitroso-R method (pH 5.5) (Ness 1965)	0–450 µg %
TIBC	Tptz-method, pH 8.5 unpublished	0–600 µg %
Cholesterol	Liebermann-Burchard (Huang et al. 1961 modif.)	0–700 mg %
β-lipoprotein	Turbidimetry (Burstein) (Kunkel units)	0–50 U
Total lipids	Phenol method (Kunkel 1948)	300–2 000 mg %
Uric acid	Uricase method (Morgenstern et al. 1966)	0–18 mg „
Phosphate-P	Phosphomolybdate without deproteinization (Richterich 1965)	0–15 mg %

Table II *The five main groups in the survey*

	No. of pats.		
	Total	♀	♂
1 Verified primary hyperparathyroidism	3	2	1
2. Probable primary hyperparathyroidism	11	11	
3. Possible primary hyperparathyroidism	6	5	1
4. Hypercalcaemia of other known cause	35	18	17
5 Hypercalcaemia of unknown cause	35	25	10
	90	61	29

ism, contains patients with only a few increased serum calcium and decreased serum phosphorus values and with incomplete case histories.

The three above mentioned groups, verified, probable and possible primary hyperparathyroidism, comprise only 20% of the total number of patients. In all three groups women predominate.

4. In hypercalcaemia of know cause other than primary hyperparathyroidism the sex distribution is equal. In this group it has been possible to trace the cause of hypercalcaemia to malignant diseases, the influence of certain drugs and to thyrotoxicosis.

5. The patients with hypercalcaemia of unknown cause

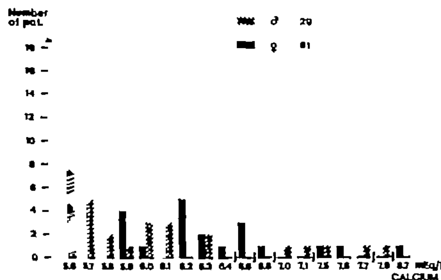


Fig 1 Distribution by sex and highest recorded serum calcium value.

suffer from diseases which are not known to induce hypercalcaemia or they have not, as far as we know used drugs which are known to influence the calcium homeostasis.

In the whole investigation, patients with only moderate increase in serum calcium predominate, although there seems to be bimodal distribution (Fig. 1). The highest value recorded is 8.7 mEq/l. Women predominate in all groups except in those with extremely high values.

Fig. 2 shows that most of the patients are rather old. Two patients under 20 years of age have, however, been found. Both had rheumatoid arthritis and only slightly increased serum calcium. The age and sex distribution may be compared to that of the population in the county of Jämtland (Fig. 3).

Verified, probable and possible hyperparathyroidism

Distribution according to calcium level (Fig. 4) and age (Fig. 5) shows that patients with verified and probable hyperparathyroidism have high calcium levels, while those classified as possible hyperparathyroidism have only moderately elevated serum calcium levels and belong to the oldest age groups. It seems likely that the combination of old age and only moderately elevated serum calcium in group 3 may explain why the clinical investigation has not been followed up so that might classify patients in one of the first two groups.

Most of the patients in groups 1, 2 and 3 make up the second peak in the bimodal distribution curve (Fig. 1).

Hypercalcaemia of other known cause

Hypercalcaemia caused by other reasons than hyperparathyroidism has been found in 35 patients, i.e. 40% of the whole survey (Table II).

In this group hypercalcaemia is most commonly caused *by drugs*. In drug-induced hypercalcaemia women predominate (Fig. 6), the increase in serum calcium is moderate (Fig. 7) and the dominating drugs are thiazides (Table III).

Hypercalcaemia caused by malignant diseases is commonly pronounced and seems to affect men more than women (Figs. 6 and 7). The diseases found are carcinoma of the lung (3 cases), kidney (3 cases), breast

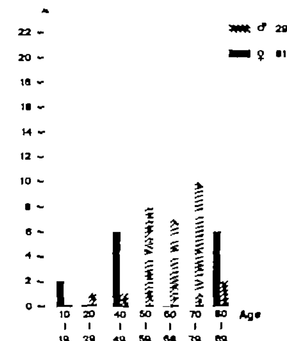


Fig. 2 Age distribution of patients with hypercalcaemia.

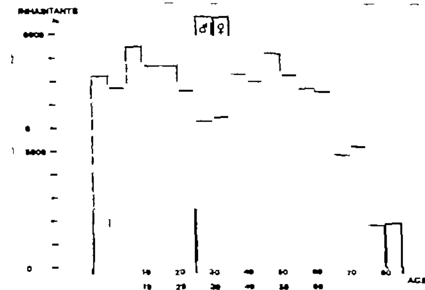


Fig. 3. Age and sex distribution of the population of the county of Jämtland.

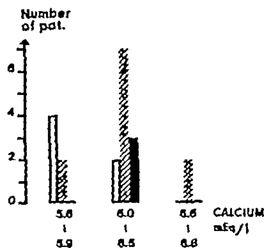


Fig. 4

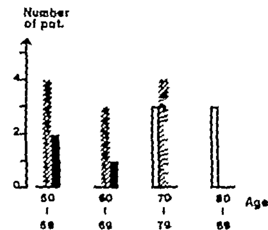


Fig 5

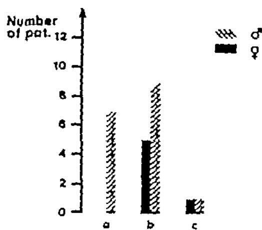


Fig 6

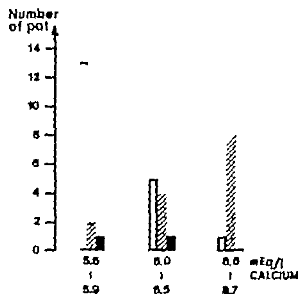


Fig 7

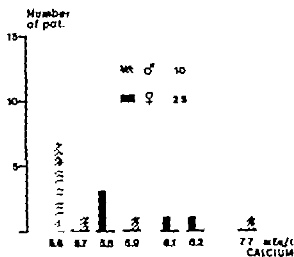


Fig. 8

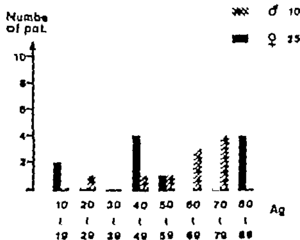


Fig 9

(2 cases), oesophagus (2 cases) and stomach (1 case). The haematology is represented by multiple myeloma (2 cases) and acute leucaemia in combination with parathyroid adenoma (1 case). Many of these patients had no signs of metastases.

Hypercalcaemia of unknown cause

The fifth main group, hypercalcaemia of unknown cause, covers about 40% of the survey (Table II). Serum calcium is as a rule moderately elevated and women in the upper age groups predominate (Figs. 8 and 9).

The distribution curve is suggestive of that seen in thiazide therapy (Fig. 7) and in possible hyperparathyroidism (Fig. 4). It is also somewhat similar to the right hand extreme of Gaussian curve and therefore should contain few normal cases with high serum calcium.

CASE REPORTS

The fact that the whole test battery is always run on the serum samples sent to the laboratory has in many cases made it possible to classify the nature of hypercalcaemia. In other cases the repeated calcium determinations have revealed quite unexpected developments. This is illustrated by a few case reports.

Cases 1 and 2

In thiazide-induced hypercalcaemia the serum calcium is only moderately increased and decreases slowly after withdrawal of the drug (Figs. 10 and 11). Fig. 11 also shows the increase in uric acid caused by thiazide.

Case 3

Fig. 12 illustrates a patient with hypernephroma without known metastases where serum calcium becomes normal after nephrectomy.

Fig. 4. Distribution according to serum calcium level for groups 1, 2 and 3. ■ = verified, ▦ = probable, □ = possible primary hyperparathyroidism.

Fig. 5. Distribution by age for groups 1, 2 and 3. Symbols as in Fig. 4.

Fig. 6. Sex distribution of patients with hypercalcaemia due to other reasons than primary hyperparathyroidism (*a* drugs, *b* malignant diseases and *c* thyrotoxicosis, 19, 14 and 2 patients, respectively).

Fig. 7. Calcium level in patients with hypercalcaemia caused by drugs (□), malignant diseases (▦) and thyrotoxicosis (■).

Fig. 8. Serum calcium level in patients with hypercalcaemia of unknown cause.

Fig. 9. Age distribution in patients with hypercalcaemia of unknown cause.

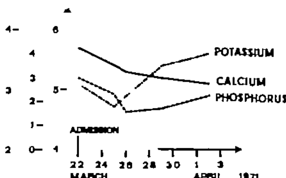


Fig. 10. Thiazide-induced hypercalcaemia in a patient with diabetes mellitus.

Case 4

Fig. 13 illustrates a patient with fulminant leucaemia with quite unexpected and pronounced increase in serum calcium. At autopsy a parathyroid adenoma was found.

Case 5

A patient with an uncomplicated cancer of the stomach became seriously ill a few days after operation, with abdominal pains and vomiting. He later died suddenly. Autopsy revealed fatal lung embolism but no bone metastases. Fig. 14 shows the sudden and unexplained increase in serum calcium and decrease in serum phosphorus.

DISCUSSION

In this investigation we have studied patients with serum calcium of 5.6 mEq/l or more. The normal value of serum calcium (mean ±2) for blood donors at this hospital is 4.97 ± 0.41 mEq/l. During the time of this investigation we have observed one blood donor out of 3 000 with a serum cal-

Table III. *Drugs causing hypercalcaemia*

	No. of pats.		
	Total	♀	♂
Thiazides (bendroflumethiazide, polythiazide and chlorthalidone)	14	10	4
Calcium (L)+thiazide	2	1	1
Calcium (L)	1	1	
Calcium-D-vitamin (ren. transplant.)	1		1
Calciferol	1		1
	19	12	7

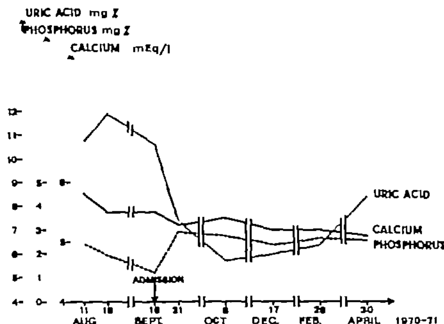


Fig. 11 Thiazide-induced hypercalcaemia in a patient with hypertensive heart disease.

cium of more than 5.5 mEq/l. This implies that the arbitrary limit of the investigation excludes borderline cases. This is also shown by the fact that, during the period of the investigation, one patient with a maximum serum calcium of 5.5 mEq/l was diagnosed as having hyperparathyroidism and verified by operation and histopathological examination.

The true frequency of diseases causing hypercalcaemia is, of course, rather difficult to estimate. However the frequency of hypercalcaemia (5 6 mEq/l or more) found in this study—100/year/100 000—may indicate that the true frequency in the population examined may be 1–2‰/year The frequency of hyperparathyroidism in this investigation seems to accord with that found by others (1). It might be of interest to note that drugs of different kinds, especially

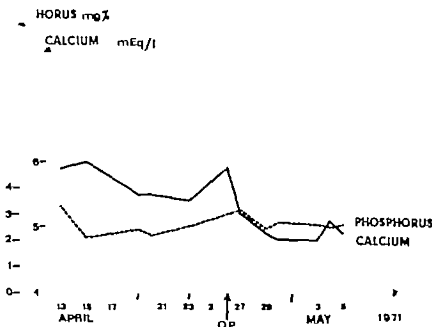


Fig. 12. Calcium and phosphorus levels before and after nephrectomy in patient with hypernephroma.

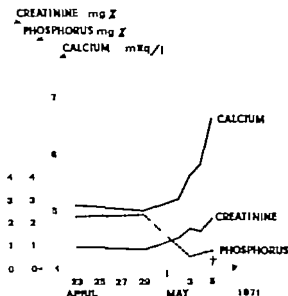


Fig. 13. A patient with acute leucaemia, progressive hypercalcaemia and parathyroid adenoma.

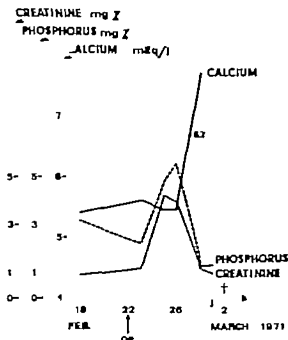


Fig. 14. A patient with malignant tumour of the stomach and unexplained progressive hypercalcaemia.

thiazides (2, 3 4 5 6, 7 8, 9 11), are the most common cause of hypercalcaemia, closely followed by malignant diseases and hyperparathyroidism. Further investigations are planned for the large group with hypercalcaemia of unknown cause.

The screening procedure used in this hospital has, in our experience, definite advantages (10). This is illustrated by the above mentioned findings of patients with hypercalcaemia. The screening procedure also makes it possible to detect sudden changes in calcium metabolism, as illustrated by the case reports (e.g. Figs. 13 and 14). Further investigation of such cases might be of importance in order to obtain a more complete understanding of calcium metabolism.

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Acta

Acta med. scand. Vol. 193 pp. 175–179 1973

HYPERCALCEMIA IN ADRENOCORTICAL INSUFFICIENCY

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Abstract. Serum calcium has been examined in 4 patients with Addison's disease and in 5 with symptoms of manifest adrenocortical insufficiency after adrenalectomy for Cushing syndrome. In each group hypercalcemia was found in 3 patients, and the incidence of hypercalcemia was the same as that of hyponatremia. Hypercalcemia alone occurred in 3 patients, as also did hyponatremia. Hypercalcemia without other disturbances of serum electrolytes led, in one of the patients, to surgical exploration of the neck in the search for parathyroid adenoma. Determination of serum calcium should be routine procedure in patients with suspicion of adrenocortical insufficiency and this diagnosis should not be forgotten in cases with hypercalcemia. Possible mechanisms for the hypercalcemia in adrenocortical insufficiency are discussed.

Hyponatremia and hyperkalemia are considered the most consistent and typical disturbances of serum electrolytes in adrenocortical insufficiency. It is, however, not generally known that hypercalcemia also may be found if searched for.

Our interest in hypercalcemia as a sign of adrenocortical insufficiency arose in connection with an operation on one of our patients for Cushing's syndrome.

CASE REPORT

A 55-year-old woman with typical Cushing's syndrome underwent unilateral adrenalectomy in Sept. 1969 because of a large adrenocortical adenoma. She was given corticosteroids, and the operation was uneventful. Cortisone was gradually withdrawn from the 5th postoperative day resulting in hypotension, asthenia, drowsiness and anorexia with vomiting. Hypercalcemia was found repeatedly, values ranging from 11.5 to 13.2 mg/100 ml, while serum sodium, potassium and chloride were within the normal range.

We considered it possible that the patient might also have an active adenoma in her parathyroids, although the hypercalcemia was not present before adrenalectomy and hypercalcemia in hyperparathyroidism is usually not corrected after treatment with corticosteroids, as was the case in our patient. The diagnosis was supported by subnormal values of inorganic phosphate in serum and lowered tubular reabsorption of phosphate (TRP 50 and 73%).

The parathyroid glands were not identified by surgical exploration of the neck in May 1970. The patient still needs corticosteroids to prevent hypercalcemia, but is without symptoms on cortisone, 25 mg/day.

It is now our opinion that the hypercalcemia was due to adrenocortical insufficiency, the remaining adrenal gland being suppressed by long lasting autonomous corticosteroid secretion from the removed adenoma. Stimulation with ACTH did not increase the very low basal values for plasma 11-OHCS.

In order to evaluate the frequency of hypercalcemia and its relation to other disturbances of serum electrolytes in adrenocortical insufficiency, we have reviewed the patients admitted to our department for Addison's disease in the 10-year period 1961–70, and the patients treated for Cushing's syndrome.

MATERIAL AND METHODS

In the above mentioned period we have diagnosed Addison's disease in 19 and Cushing's syndrome in 20 patients. Some details from the latter are given below.

Analyses of serum calcium and inorganic phosphate, in contrary to serum sodium, potassium and chloride, were not routinely performed in these patients up to July 1969. From that time serum calcium and phosphate have been measured with SMA 12/60 (Autoanalyzer) (7) in all patients admitted to our department. This method gives more accurate and precise information than the methods used before. Until 1964 serum calcium was determined by EDTA titration (5), in the years 1964–69 by a colorimetric method (16). Before July 1969 inorganic phosphate was measured by the Fiske-Subbarow procedure (3). The normal ranges for the values (serum calcium 8.5–10.5 mg/100 ml, inorganic phosphate 2.5–4.8 mg/100 ml) are, however, the same although different methods were employed.

Table I. Frequency of pathological serum electrolytes in 19 patients with Addison's disease and in 8 with symptoms of adrenocortical insufficiency after adrenalectomy for Cushing's syndrome

Pathological. Na < 136 mEq/l, K > 5.0 mEq/l, Cl < 97 mEq/l, Ca > 11 mg/100 ml, P > 4.8 mg/100 ml

	Addison's disease					Adrenalectomized patients				
	Na	K	Cl	Ca	P	Na	K	Cl	Ca	P
Patients examined	19	19	16	4	4	8	8	8	5	4
Patients with pathological values	15	11	7	3	2	6	4	3	3	1

In the present investigation we have only considered a serum with calcium > 11.0 mg/100 ml as hypercalcemic.

Serum chloride is determined as described by Cotlove (2). Serum sodium and potassium are measured with a Beckman automatic flame photometer with internal lithium standard. The normal values are: sodium 136–145, potassium 3.5–5.0, chloride 97–107 mEq/l.

RESULTS

Addison's disease

In all 19 patients serum sodium and potassium were determined at a time when subjective symptoms of adrenocortical insufficiency were present. In the same situation serum calcium and inorganic phosphate were determined in 4 patients.

The results are shown in Table I. For some patients several analyses were obtained. The results are recorded as pathological if only one analysis was found to be abnormal although other analyses of the same parameter at other times may have been within the normal range.

Hyponatremia was present in 15 of the 19 patients on one or more occasions. In the same 15 patients 10 had also hyperkalemia. In the 4 patients in whom serum calcium was determined, 3 had hypercalcemia; serum sodium and potassium were normal in 2 of these, while the third also had hyponatremia and hyperkalemia. In the fourth patient a normal serum calcium was accompanied by a marked hyponatremia (122 mEq/l) and hyperkalemia (7.2 mEq/l).

Inorganic phosphate was moderately increased in 2 patients with hypercalcemia, while it was normal in the remainder.

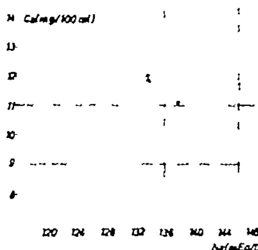


Fig. 1. Serum calcium in relation to serum sodium in patients with adrenocortical insufficiency (22 analyses from 9 patients). Serum calcium and sodium examined in the same blood sample. O = patients with Cushing's syndrome, x = patients with Addison's disease.

Cushing's syndrome

Serum calcium and inorganic phosphate were determined in 15 of the 20 patients before surgical treatment. The values were within the normal range in all.

Pathoanatomical diagnoses and kinds of treatment were as follows. Unilateral adrenalectomy was performed in 4 patients with adrenocortical carcinoma and in 2 with adrenocortical adenoma. In the 14 patients with bilateral adrenocortical hyperplasia, 2 underwent unilateral adrenalectomy accompanied by pituitary irradiation, subtotal adrenalectomy was performed in 6 and total adrenalectomy in 5 patients. One patient with ectopic ACTH secretion from a carcinoid tumour was not operated on.

None of the patients operated on for adrenocortical carcinoma developed hypercalcemia. Indeed 2 patients, dying with endocrine active metastases, had a marked hypocalcemia in the last months of their life. One patient who developed hypercalcemia after unilateral adrenalec

tomy for a cortical adenoma is referred to above. Seven of the other patients were later readmitted with manifest symptoms of adrenocortical insufficiency 4 after *subtotal*, 3 after *total* adrenalectomy Serum calcium was determined in 4 of them, and hypercalcemia was found in 2, one of whom was also hyponatremic, while serum sodium was normal in the other. Two patients had hyponatremia without hypercalcemia.

The occurrence on one or more occasions of abnormal serum electrolytes in the 8 patients operated on for Cushing's syndrome and who developed manifest symptoms of adrenocortical insufficiency is summarized in Table I.

Hyponatremia was the most frequent abnormality observed, but the relative incidence of hypercalcemia was almost the same. Hypochloremia and hyperkalemia were not seen without coexistent hyponatremia.

Fig. 1 shows the relationship between serum sodium and calcium in the 9 patients with adrenocortical insufficiency (Addison's disease 4 Cushing's syndrome 5) in whom both parameters were analysed in the same blood sample. In 6 patients only one analysis of the two parameters was made. More than one analysis was performed in 3 patients, altogether 16 analyses, and in these hypercalcemia was found repeatedly Hypercalcemia was found in 17 samples from 6 patients; in 12 of these samples serum sodium was normal. Nine samples revealed hyponatremia in these serum calcium was normal in 4. Low serum sodium was accompanied by hypercalcemia in 5 samples.

DISCUSSION

In 5 patients with symptoms of adrenocortical insufficiency after operation for Cushing's syndrome we found hypercalcemia in 3. We also found hypercalcemia in 3 of 4 patients with Addison's disease in whom serum calcium was determined. The hypercalcemia and the subjective symptoms of adrenocortical insufficiency were corrected by corticosteroids.

It seems therefore that hypercalcemia is a frequent sign of adrenocortical insufficiency There is reason to emphasize that hypercalcemia may be the only abnormality found in serum electrolytes.

When including repeated analyses from some patients, hypercalcemia was more frequently diagnosed than hyponatremia in the present investigation when both sodium and calcium were analysed in the same blood sample. The number of hypercalcemic patients was, however the same as the hyponatremic. Among the 9 patients both occurred in 6. Hypercalcemia alone was diagnosed in 3 patients, as also was hyponatremia.

Serum potassium and chloride were less sensible indicators of the adrenocortical function than sodium and calcium.

In contrast to the hypercalcemia seen in hyperparathyroidism, the hypercalcemia in adrenocortical insufficiency is often accompanied by high inorganic phosphate in serum (9 13 14) We found serum inorganic phosphate to be moderately increased in 3 of the 8 patients in whom it was determined, while it was within the normal range in the remainder

Hypercalcemia in patients with Addison's disease was first described by Loeb (10) in 1932. In 1963 Walser et al. (19) collected 15 patients with Addison's disease from the literature and presented 16 of their own cases in whom serum calcium and sodium were determined from the same blood sample or at least on the same day Like us, they found the frequency of hypercalcemia to be nearly identical with that of hyponatremia. There was no correlation, either positive or negative, between these two parameters, which also occurred separately with about the same frequency Walser et al. made corresponding observations in 25 adrenalectomized dogs kept on a calcium-free diet.

Later 3 patients have been reported (13 15) in whom the symptoms were attributed to the diagnosed hypercalcemia before the final diagnosis of Addison's disease. In one of these patients (15) the suspicion of adrenocortical insufficiency arose when the patient rapidly and unexpectedly improved in the course of a test with corticosteroids employed for differential diagnosis of the hypercalcemia. In this connection it is worth remembering that the most outstanding subjective symptoms in adrenocortical insufficiency closely mimic those of hyperparathyroidism. In one of our patients a wrong diagnosis led to surgical exploration of the neck in the search for an adenoma in the parathyroids.

Sprague et al. (17) found in 40 patients, after subtotal adrenalectomy for adrenocortical hyper

plasia, that many became hypercalcemic on the attempt to withdraw corticosteroids 10–20 days postoperatively None of these were hypercalcemic before the operation. The hypercalcemia was corrected by renewed treatment with corticosteroids.

While there is no disagreement that the hypercalcemia seen in adrenocortical insufficiency is due to deficient corticosteroid secretion, it is more questionable by what mechanism the hypercalcemia is produced.

Several hypotheses have been proposed in explanation. The topic has been thoroughly discussed by Walser et al. (19), who made serious objections to the theories that increased parathyroid gland activity or increased sensitivity to vitamin D due to loss of corticosteroid antagonism could explain the hypercalcemia.

Cortisone is shown to inhibit the active transport of calcium by the isolated gut (6). The fact that adrenalectomized dogs were equally hypercalcemic whether the diet was calcium-free or not (19) does not support the theory of increased intestinal absorption of calcium as the cause.

From balance studies in adrenalectomized dogs Walser et al (19) suggest the following mechanisms as responsible for the hypercalcemia. Firstly hyperproteinemia associated with hemoconcentration induced an increase in protein-bound calcium. Secondly an increased affinity of plasma protein for serum calcium was found. This was partly attributed to the accompanying hyponatremia. They also found an increase in filterable calcium complexes, while the concentration of free calcium ions was normal.

They do not, however deny a renal mechanism as responsible for the hypercalcemia. Many observations point to a deficiency or excess of corticosteroids as an important factor in the renal tubular handling of calcium.

The renal calcium excretion is high in Cushing's syndrome and decreases after adrenalectomy (1). We found hypocalcemia in . of our patients with Cushing's syndrome and adrenocortical tumour The mechanism may however be complex and partly attributable to the accompanying hypoproteinemia.

Both in humans and animals long-time administration of corticosteroids increases the renal excretion of calcium (8 11 14 18) Laake (8) showed this to be due to decreased tubular reabsorption of calcium. Studies by Massry et al. (11) and Suki et al. (18) imply that the corticosteroid effect on tubular reabsorption of calcium is not due to a direct effect on renal tubules, but is mediated rather by the induced increase in extracellular fluid volume. Extracellular expansion decreases sodium and calcium reabsorption in proximal tubules, in which segment the handling of these ions seems to be closely linked. Such a decrease in proximal tubule reabsorption enhances the delivery of sodium and calcium to the distal tubules, where the corticosteroids may promote sodium reabsorption without a direct effect on calcium transport. The net result would be an increased excretion of calcium. The increase in renal calcium excretion in animals on corticosteroids was lacking when sodium was omitted from the diet (18).

It would not then be surprising if corticosteroid deficiency was accompanied by diminished renal excretion of calcium. Studies in renal handling of calcium in adrenocortical insufficient patients are lacking. Walser et al. (19) however found the tubular reabsorption of calcium to be excessive in adrenalectomized dogs.

It is shown that infusion of angiotensin decreases the renal calcium excretion and produces a significant increase in serum calcium (4). This is of interest since plasma renin activity is known to be increased in patients with Addison's disease (1) The importance of this for the hypercalcemia in adrenocortical insufficiency is, however quite uncertain.

CONCLUSION

Hypercalcemia seems to be a frequent sign of adrenocortical insufficiency and it may be the only abnormality found in serum electrolytes Determination of serum calcium should therefore be included in the analyses when adrenocortical insufficiency is suspected, and also for the control of adequate corticosteroid substitution in such patients.

Hypercalcemia is often a diagnostic challenge, and adrenocortical insufficiency should be considered as a possible although rare reason.

The pathogenesis for the hypercalcemia may be complex, but increased renal tubular reabsorption of calcium is likely to be of importance

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Acta med. scand. Vol. 193 pp. 181–188 1973

EXTRACORPOREAL IRRADIATION OF THE BLOOD

Clinical Results after Necro-kidney Transplantation

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Abstract. The clinical results of necro-kidney transplantation after extracorporeal irradiation of the blood (ECIB) are presented. The patient material consists of 91 consecutively transplanted patients; only first necro-kidney grafts are considered. Thirty-seven patients received ECIB before as well as immediately following transplantation (group I), 54 patients did not receive ECIB (group II). The clinical results are evaluated from the number and severity of rejection episodes, the graft survival and the clinical rank. In group I the total number of rejection episodes was significantly reduced within the first 2 months ($p < 0.01$) and the number of patients without rejection during a total observation period of 26 months was significantly higher ($p < 0.01$). The graft survival after 6 months was increased by 25% ($p < 0.05$); after 12 months the number of observations was too small to permit a reliable analysis. The clinical rank after 6 months was better in group I ($p < 0.05$) but indistinguishable after 12 months. There was no significant difference between the two groups regarding the number of complicating severe infections caused by bacteria, virus or fungi.

To-day prevention of homograft rejection is mainly based on tissue typing and on immunosuppressive therapy. The superior outcome in HL A identical siblings and the good correlation between clinical results and HL A typing in related donors indicate the primary importance of the HL A antigens. But it is well known that other systems may influence the results, since immunosuppressive therapy is necessary also when HL A identical sibling donors are used (10, 12).

The immunosuppressive agents commonly used in the clinic, corticosteroids and cytotoxic drugs, are immunologically non-specific and have widespread harmful effects on tissues not belonging to the immune organ. Methods directed to selective damage of the lymphocytes (thoracic duct drainage, antilymphocytic globulin (ALG), and extracorporeal irradiation of the blood (ECIB) or the lymph (ECIL)), have less widespread side-effects, although they cannot be regarded as being immunologically specific. The ideal goal is to achieve a state of selective unresponsiveness to the HL-A antigens of the donor, leaving the immunological responsiveness to other antigens intact. Specific immunological tolerance and immunological enhancement belong to this category but are not yet available in the clinical transplantation situation.

Experimental works by Cronkite and Chanana (5) and their co-workers (2, 3, 7) have clearly shown that ECIB improves skin and kidney graft survival in calves and goats. In human kidney transplantation ECIB has been used before transplantation in Gothenburg since 1965 and in Copenhagen since 1968 (15, 16, 22). It was shown that ECIB decreases the number and severity of rejection episodes within the first 6 weeks after transplantation. The late results on graft survival are, however, still unclarified.

The purpose of this study is to present early and late results of ECIB applied before as well as in the immediate period following necro-kidney transplantation.

MATERIAL AND METHODS

Patients. In the period from Dec. 10th, 1968 to Oct. 1st, 1971, a total of 110 necro-kidney transplantations have been performed at Rigshospitalet. Thirteen of these transplantations were retransplantations and are excluded because the group is very small, and since analyses of large materials indicate that second grafts have a poorer prognosis (14). Among the remaining 97 consecutive first necro-kidney grafts a further six cases were excluded. Two patients received ECIB prior to transplantation at

Table I *Clinical data of the 91 patients (median values and range)*

Group	No. of pats.	Observation time (mo.)	Age (y)	Sex ( )	Sex (♂)	Primary kidney disease: Glomerulonephritis	Pyelonephritis	Various other	Duration of uraemia (mo.)	Duration of dialysis (mo.)	Not dialysed	No. of blood transfusions
I	37	8.5 (0.5–25.5)	40 (21–59)	21	16	17	17	3	18.5 (2–84)	10.5 (1–63)	3	36 (6–273)
II	54	4.0 (0.5–24.5)	47 (17–58)	29	25	15	32	7	24.0 (1–96)	3.0 (1–36)	13	13.4 (0–109)

another centre and were retransferred to this centre shortly after transplantation. Four patients received ECIB only immediately following transplantation. This small material does not permit an evaluation of the possible value of this type of treatment.

Sex and age. The median age of the remaining 91 patients, 50 women and 41 men, was 45 years (range 17–59).

The primary kidney disease was glomerulonephritis in 3[illegible], pyelonephritis in 49 and various other diseases in 10 patients. The diagnosis of the primary kidney disease was based on clinical and histological criteria. Kidney tissue for histological examination was obtained by renal biopsy and/or at bilateral nephrectomy. Histological examination was performed in 81 cases. The histological diagnosis was definite in 41 of the kidneys examined, while 40 were maximally contracted kidneys in which exact histological diagnosis could not often be obtained.

Tissue typing. All recipients and donors were tissue typed before transplantation at the Tissue Typing Laboratory of Rigshospitalet by L. Staub Nielsen and A. Svejgaard according to the methods of Kissmeyer-Nielsen and Thorsby (8). The match grades are presented as A, B, C, D, E and F matches according to generally accepted criteria (9). The worst possible match is used, i.e. serologically non-identified antigen(s) in the donor are classified as incompatible. Among the 91 patients there were 6 A, matches, 42 C matches, 40 D matches and 3 E matches. In 4 recipients (2 C and D matches) antibodies active against the donor HL-A antigen(s) were demonstrated.

The donor kidneys were all necro-organs, 83 provided from Scandiatransplant and 8 from Eurotransplant. The median age of the donors, 30 women and 61 men, was 30 years (range 10–65). The warm ischaemic period, defined as the time (min) from cardiac arrest or clamping of the renal artery to start of the cooling perfusion was median 15 min (range 1–90). The cold ischaemic period, defined as the time (hours) from start of cooling perfusion to recirculation in the recipient was median 6 hours (range 1–18).

The transplantations were performed by conventional technique, the donor kidney being placed in the contralateral iliac fossa. Bilateral nephrectomy was carried out before transplantation in 16 patients, in connection with transplantation in 46, and after transplantation in 3. Twenty-six of the 91 patients were not nephrectomized. The immediate postoperative function of the donor kidney was good in 54 of the 91 patients. In 27 patients there was delayed diuresis with need for haemodialysis within the first 3 weeks. In 8 patients the donor kidney never functioned (2 renal vein thrombosis, 6 acute rejections). Another 2 patients died from intercurrent diseases before function of the donor kidney.

The conventional immunosuppressive therapy after transplantation consisted of azathioprine (Imurel®) and prednisone: the daily dose of Imurel/kg b.w. was on an average 1.6 mg and was kept approximately constant. The dose of prednisone was higher following transplantation and gradually reduced. Rejection crises were treated with increased doses of steroids. Twenty-three patients received local graft irradiation with 150 rads three times within the first week after transplantation.

ECIB. Thirty-seven of the patients received ECIB before transplantation (group I), 54 did not receive ECIB before transplantation (group II). The technique of ECIB has been described elsewhere (20). Nine of the 37 patients who received ECIB before transplantation were given a median transit dose of 100 rads (range 100–120), a total number of blood volumes irradiated of 185 (range 88–482) and a total radiation dose of 19 850 rads (range 9 920–39 860). Twenty-eight of the 37 patients received a median transit dose of 340 rads (range 160–650), a total number of blood volumes irradiated of 130 (range 4–303) and a total radiation dose of 50 000 rads (range 7 170–82 200). ECIB was terminated on an average 2.5 months prior to transplantation (range 1 day to 14 months). At transplantation the lymphocyte concentration in the blood was about 430/µl (range 250–770).

After transplantation the ECIB pretreated patients all received ECIB in the immediate postoperative period. The duration varied from 3 to 115 hours, the average transit dose was between 44 and 660 rads and the total radiation dose between 1 600 and 88 300 rads. During clinical rejection episodes 7 of the 54 patients who were not pretreated with ECIB received ECIB as a supplement to increased prednisone dosage.

The clinical results are evaluated from the number and severity of rejection crises, the graft survival, and the clinical rank.

Early rejection. In patients with initial kidney function the diagnosis of rejection was established by a significant reduction of 24-hour endogenous creatinine clearance in two consecutive determinations. In patients with anuria the diagnosis was established by explorative surgery and biopsy. In both groups every attempt was made to exclude other causes of reduction in kidney function by

Table II. *Data of the 91 donors and the match grades (median values and range)*

Group	No. of donors	Age of donors (y)	Match grade A	C	D	E	F	HL-A 1.8	Ischaemic time Warm (min)	Cold (h)	Postoperative function Immediate diuresis	Delayed diuresis necessitating dialysis	Never functioned
I	37	30 (14–65)	1	13	21	2	3	8	11 (1 90)	6 (1/2–14)	20	13	4
II	54	30 (10–62)	5	29	19	1	1	12	19 (1 50)	6 (1 1/2–18)	34	14	6

means of isotope nephrography i. urography or pyelography and angiograms. Because symptoms such as increase in BP and proteinuria might be caused by various other complications (stenosis of the vascular anastomosis, temporary overloading, infections, patient's own diseased kidneys not removed), the presence of, or changes in, these parameters without concomitant reduction of renal function was not considered diagnostic.

Rejection episodes were graded in three stages: 1) Fully reversible, i.e. creatinine clearance ended up with value normal for the age and sex of the donor 2) Partially reversible, i.e. creatinine clearance ended up with value lower than might be expected for the age and sex of the donor 3) Fulminant and irreversible, followed by graftectomy

Late graft failure as indicated by gradual reduction in kidney function months to years after transplantation, might be caused by host versus graft reaction, glomerulonephritis in the grafted kidney surgical complications, infections, and perirenal fibrosis. In the analysis of the material late graft failures appear from the clinical rank and the graft survival.

The graft survival analysis is based primarily on all causes of death of the patient or loss of the graft. Secondly the causes of loss of the graft or death of the patient were divided into immunological and non-immunological failures. Immunological failures" comprise acute rejection and complications (septicaemia, intestinal bleeding) most probably induced by heavy immunosuppressive therapy instituted because of severe rejection episodes. Included are also uraemia and/or cardiovascular complications caused by late graft failures. "Non-immunological failures comprise death of patient or loss of graft caused by complications unrelated to rejection and without histological evidence of homograft reaction. These causes include surgical complications (renal vein or artery thrombosis, infections originating from ureteral leakage or perirenal haemorrhage), hepatic coma due to hepatitis transmitted during dialysis and cardiovascular or embolic complications seen mostly in elderly arteriosclerotic recipients. Two cases of haemorrhage from chronic duodenal ulcer are also included.

Clinical result. The follow-up clinical results are graded according to Termaki (11) and estimated 6 and 12 months after transplantation.

Statistical methods used are: Student's *t*-test, Fisher exact probability test, χ^2-test, and life table analysis (6, 10).

RESULTS

The clinical data of the recipients, the donors, and the immunosuppressive therapy after transplantation, are presented in Tables I, II and III for the ECIB pretreated group I and the non-ECIB pretreated group II. The two groups were comparable considering the presented data, except for the match grades, which were better in group II ($p < 0.05$) the number of blood transfusions before transplantation, which was greater in group I ($p < 0.01$) the prednisone dosage on day 0–21 following transplantation, which was higher in group II ($p < 0.01$) and the number of patients who received graft irradiation (21 in group II and 2 in group I)

The two groups differ also in regard to the centres where the patients were treated before transplantation 33 of the 37 patients in group I were treated at Rigshospitalet, 4 at other centres, 19 of the 54 patients in group II were treated at Rigshospitalet, 35 at other centres ($p < 0.01$). A graft survival analysis was, therefore, undertaken

Table III. *Immunosuppressive therapy after transplantation (median values and range)*

Group	ECIB	Graft irradiation	Prednisone day 0–21 (mg/kg b.wt)	Imuran day 0–21 (mg/kg b wt)
I	37	2	1 1 (0 5–5 3)	1 7 (0 4–3 0)
II	7*	21	2 4 (0.5–10 6)	1 9 (0 6–3.1)

Only at rejection crises.

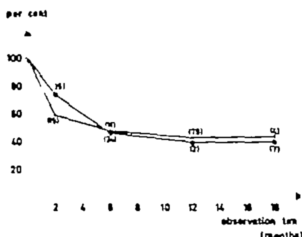


Fig. 1. Cumulative graft survival for the non-ECIB pretreated patients from Rigshospitalet (●—●) and the non-ECIB pretreated patients from other centres (O—O). Number of patients at risk given within parentheses.

between the non ECIB pretreated patients from Rigshospitalet and the non-ECIB pretreated patients from other centres (Fig. 1). The cumulative graft survival was identical among the 19 patients from Rigshospitalet and the 35 patients from other centres.

Rejection episodes. In group I there were 14 patients with 17 rejection episodes and in group II 37 patients with 49 rejection episodes. Table IV shows all rejection episodes within the first two months for the two groups. In group I there was

Table IV. *Number and grade of rejection episodes within the first 2 months after transplantation*

Group	No. of pats. Without rejections	With rejections	Total no. of rejections	Grade 1	2	3
I	24	7	7	3	3	1
II	14	32	41	14	17	10

a significant reduction in rejection episodes ($p < 0.01$). Among rejecting patients there was, however, no difference in the severity of rejections as estimated by their grade ($p > 0.1$). Fig. 2 shows the cumulative fraction of patients within the two groups presenting no rejection episodes at all within the whole observation period of 26 months. The difference between the two groups is significant ($p < 0.01$).

The cumulative graft survival. Table V shows the immunological and non-immunological causes of death or loss of the graft in groups I and II. The cumulative graft survival, considering all failures and immunological failures alone, is shown in Fig. 3 and Table VI. Considering all failures the fraction surviving after 6 months was higher in group I than in group II ($0.05 > p > 0.01$). After 12 months there was no difference between the two groups. Considering only immunological fail-

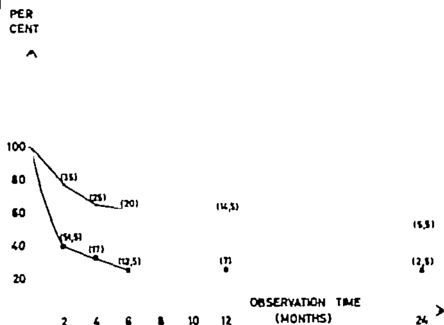


Fig. 2. Cumulative fraction of patients without rejections in group I (●—●) and group II (O—O). Number of patients at risk given within parentheses.

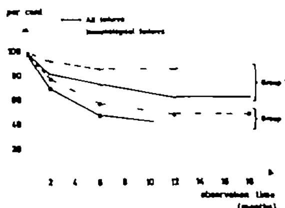


Fig. 3. Cumulative graft survival for patients in groups I and II.

ures, the difference in graft survival was significant after 6 as well as after 12 months ($p <$ 0.01) (Table VI).

The clinical rank for patients in groups I and II, 6 and 12 months after transplantation, is shown in Table VII. The number of patients classified in clinical rank I to II after 6 months was higher in group I than in group II ($p < 0.05$). After 12 months there was no difference between the two groups.

The infectious complications in groups I and II are shown in Table VIII. The bacterial infections include septicaemia or multiple abscesses. The two cases of tuberculosis, both in group I, were patients with pulmonary infiltration and positive culture of TB after transplantation and no signs or symptoms of tuberculosis before transplantation. Furthermore two patients, one in each group had tuberculosis before transplantation and were treated with chemotherapy at the time of grafting. They are not included in Table VIII. The fungous infections are severe cases with multiple foci of fungi cultivated from the blood, spleen and lungs after death. In this small material a comparison between the frequency and nature of the infectious complications revealed no statistical difference.

DISCUSSION

The ECIB treated group I and the non-ECIB treated group II are comparable with respect to sex distribution, age, primary kidney disease and the period of warm and cold ischemia of the graft.

The two groups differ in respect to the centre from which the patients were referred to Rigshospitalet for transplantation. As shown in Fig. 1 however this difference can apparently be disregarded.

The two groups also differ in certain other factors. Thus the patients in group II had received less blood transfusions before grafting, were grafted with kidneys of better histocompatibility received higher initial prednisone dosage and were more extensively treated with local graft irradiation.

Theoretically the greater *number of blood transfusions* in group I before transplantation might increase the number of rejection episodes. The present material is, however too small to demonstrate a difference between the two groups regarding the presence of lymphocytotoxic antibodies.

Table V *Causes of graft failures in groups I and II*

Group	Immunological failures: Acute rejection	Late graft failure	Septicaemia	Intestinal bleeding	Vascular thrombosis	Total
I	2	2	3			7
II	10	2	10	1	1	24
	Non-immunological failures: Septicaemia	Intestinal bleeding	Cardiovascular	Renal vein thrombosis	Hepatitis	
I	1	1	2	2	2	8
II	2	1	1	2	1	7

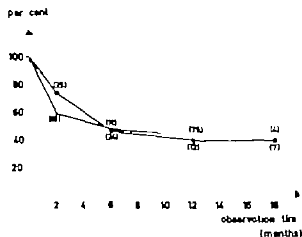


Fig 1 Cumulative graft survival for the non-ECIB pretreated patients from Rigshospitalet (●—●) and the non-ECIB pretreated patients from other centres (●—O). Number of patients at risk given within parentheses.

between the non-ECIB pretreated patients from Rigshospitalet and the non-ECIB pretreated patients from other centres (Fig. 1) The cumulative graft survival was identical among the 19 patients from Rigshospitalet and the 35 patients from other centres.

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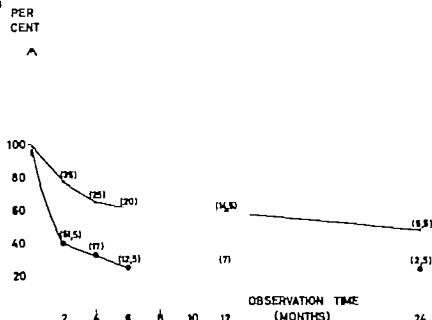


Fig. 2 Cumulative fraction of patients without rejections in group I (●—●) and group II (O—O). Number of patients at risk given within parentheses.

Table VIII. *Infectious complications*

Group	No. of pats. with infections	Total no. of infections	Septicaemia	Tuberculosis	Herpes zoster	Fungi
I	12	16	7	2	6	1
II	19	29	17	0	5	7

received a high transit dose of 380 rads and a total radiation dose of 50 000 rads.

Among methods selectively damaging the lymphocytes (13, 19) (ALG, thoracic duct drainage, ECIB, ECIL) ECIB appears to involve least side effects. The treatment is time-consuming, but if ECIB is given in connection with haemodialysis, half the time can be saved. The damage to the erythrocytes reported in in vitro studies and in leucaemia (1, 17) could not be demonstrated in uraemic patients (21). In the present small material the infectious complications were not increased after ECIB. Development of malignant diseases has not occurred among our patients, but a longer period of observation is, of course, needed for evaluation of this problem. A long-term follow-up of ECIB treated calves did not reveal malignant diseases in this species (4).

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Table I. *Design of the study*

Measurements	Therapy: Chlorthalidone	Chlorthalidone 50–100 mg		+ alprenolol/placebo (100 mg 4)		+ placebo/alprenolol (100 mg 4)	
	Weeks 0		6	12	18	24	30
BP	↑	↑↑↑	↑	↑└─┘ Per I	└─┘ Per II	↑└─┘ Per I	└─┘ Per. II
PRA	↑ (before week 0)				↑		↑
Other lab. m est.	↑		↑		↑ *n*=9		↑ =8

Only in previously untreated patients before chlorthalidone.

Most of the subjects, whereof only 2 were women, were i the age group 50–64. The duration of the hypertension varied from 1 to more than 10 years, but was usually below 5 years. Five of the subjects had eyegrounds showing FH I, eight had I–II and the remaining four FH II.

The heart volume was within normal limits in all cases except two (nos. 11 and 12), who had heart volumes around 500 ml/m²

Procedure

During an initial period of 6 weeks the chlorthalidone treatment was continued, usually ith a dose of 50 mg daily together with potassium substitution. Thereafter alprenolol was added double-blind during tw 12-week periods. Nine of 17 patients received alprenolol before placebo. Control of tablet intake showed that 95% of the tablets had been used.

BP was measured at intervals as indicated in the scheme (Table I). Measurements were made with the same mer cury sphygmomanometer cuff, always twice after 5–10 min est in the supine position and immediately after standing. lowest alues ere used for the calculations.

PRA and blood olume were measured at the end of each 12-week period, and routine laboratory determinations performed at intervals as indicated in the scheme (Table I).

For determination of blood volume and PRA the patients were admitted to the hospital in the morning. After having taken their morning tablet of alprenolol/placebo, blood volume determination according to Sjöstrand was done, with the patient resting 50 min in the supine position. At the end of this period an indwelling catheter was introduced in cubital vein and 20 ml blood for PRA determination was taken in supine position and after 15 and 30 min walking slowly around in the laboratory (erect PRA).

The blood was kept cool in icewater centrifuged in cold centrifuge and the plasma stored in a deep-freezer until processed. PRA was determined according to Boucher et al. (3) with slight modification (6). The supine value and the highest value in erect position were used for the calculation, because of ariations in the time course of renin release in the erect position. With the above standard procedure the normal values in our hands for normotensive subjects are: supine 105 ± 18 ng/100 ml, erect 179 ± 39 ng/100 ml ($\bar{X} \pm$ S.E.). Blood volume was determined according to the Sjöstrand alveolar CO method (7). Predicted normal values were obtained from linear regression equations.

RESULTS

Blood pressure

Alprenolol induced a significant decrease in both systolic ($p < 0.001$) and diastolic ($p < 0.01$) BP There were large individual variations. With the 3 patients on 50 mg × 4 alprenolol included, the

Table II. *Patients excluded from the study*

Pat. no.	Age (y)	Sex	Approx. duration of hypertension (y)	Supine BP	Chlorthalidone dose (mg)	Cause of interruption
18	62	♀	5	160/95	50	Extrasystole
19	61	♂	5	170/110	50	Died of reinfarction abroad
20	60	♂	15	180/110	50	Dyspnoea, angina pectoris? 1/2 h. after taking the tablets
21	60	♂	2	190/100	50	Vertigo – syncope?
22	57	♀	< 20	205/110	50	Dyspnea after full dose
23	39	♂	> 3	170/100	50	Gastrointestinal trouble

Table III. *Clinical data of the patients studied*

H = chlorthalidone, Ap = hydralazine, Ald = methyldopa

Pat. no.	Age (y.)	Approx. duration of hypertension (y.)	Preceding treatment	Supine BP 1st visit (syst. diast.)	Other diagnoses
1	68	4	None, previously diuretic	200/100	Angina pectoris?
2	65	10	H 100 mg Ap 25 mg 3	180/125	
3*	63	3	None	180/130	
4	62	5	H 100 mg Ap 25 3 Ald 250 mg 1 3	210/120	Myocardial infarction 1 y before, angina pectoris
5	64	10	H 50 mg	180/110	
6	61	8	H 100 mg Ap 25 mg 3	180/110	Angina pectoris?
7	61	2–3	Diuretic Aldomet 250 mg 3	200/115	Myocardial infarction 3 y before
8	60	10	H 100 mg Ap 25 mg 3 Ald 250 mg 3	220/120	
9	59	1 2	None	190/105	Diabetes treated ith tablets
10	60	5	H 100 mg Ald 250 mg 2–3	180/110	Angina pectoris?
11	60	10	H 100 mg Ap 25 mg 3	190/110	
12	54	1	None	180/115	
13	53	2–3	None. Diuretics later ultimately	180/120	
14	53	6	H 50 mg Ap 25 mg 3	160/110	Intermitt. claudication
15	50	1	None	190/100	
16	48	8	H 100 mg Ald 250 mg 3	160/110	
17*	30	4	H 50 mg Ald 250 mg 1	160/100	Toxicosis during pregnancy 4 y ago

Female.

mean decrease of systolic and diastolic pressure was −18 and −9 mmHg, respectively (Table IV) Systolic BP tended to be lower at the end of the alprenolol treatment compared with the be ginning (late change) but this difference was not statistically significant (Table V). During the alprenolol and the placebo periods there were no significant changes in BP

No orthostatic symptoms induced by alprenolol were observed The mean decrease in systolic BP in the erect position compared to supine position was 12 mmHg in the alprenolol period as well as in the placebo period.

Plasma renin activity

PRA during chlorthalidone treatment was increased in comparison with our normal values. Addition of alprenolol treatment significantly decreased PRA, but did not abolish the orthostatic increase in PRA (Table VI).

Correlation BP–PRA

The decrease in BP induced by alprenolol was significantly correlated with the decrease in PRA (Table VII). The highest correlation was between the decrease in systolic BP and the decrease in PRA in erect position (Fig. 1) The alprenolol-induced decrease in erect PRA also correlated with the late change in diastolic BP

Blood volume

The blood olume was significantly decreased during chlorthahdone treatment compared with predicted normal values. The alprenolol treatment did not influence the blood volume. During the double-blind crossover periods the second blood v lume determination was significantly decreased compared with the first, indicating a gradual decrease in blood volume during chlorthahdone treatment (Table VIII).

Correlation blood volume—PRA

During the placebo period there was an in erse but not significant correlation between blood olume and PRA During the alprenolol period there was a direct correlation, which was statistically significant when correlating erect PRA with blood volume (Table IX).

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Table VIII. *Blood volume during placebo and alprenolol periods*

	Predicted[a] values	Placebo period	Alprenolol period	1st determination	2nd determination
$\bar{x}$	6.2	5.6	5.6	5.8	5.4
S.E.	0.3	0.2	0.3	0.3	0.2
	17	17	17	17	17
p	<0.05			<0.01	

[a] See Methods.

Table IX. *Correlation between blood volume and PRA*

Independent variable Blood volume (ml/kg)	Dependent variable Supine PRA	Erect PRA
Placebo period	$r=-0.14$ $p>0.05$	$r=-0.25$ $p>0.05$
Alprenolol period	$r=0.29$ $p>0.05$	$r=0.54$ $p<0.05$

suppress the orthostatic increase in PRA. This may be due either to a direct stimulation on renal adrenergic receptors or to the different hemodynamic effects of the two drugs. The difference between various β-adrenergic blocking agents in this context is also exemplified by a study by Alexander et al. (1) who found that propranolol seems to be more effective than oxprenolol in suppressing PRA in rats. Furthermore it cannot be excluded that other mechanisms than the renal nerves are of importance for the orthostatic release of PRA.

The correlation between the effects induced by alprenolol on BP and PRA may suggest that the suppression of the renin-angiotensin-aldosterone system plays a role in the hypotensive effect of β-adrenergic blocking agents. The possibility that the BP as well as the PRA response to alprenolol treatment are correlated to a common variable, such as the "lability" of the sympathetic nervous system in the individual patient, can however not be excluded. Furthermore the hypotensive effect of β-adrenergic blocking agents in this study is of the same magnitude as in patients without other hypotensive therapy (11 17). As these patients have normal and sometimes even subnormal PRA, suppression of the renin-angiotensin system in such situations may be expected to play only a minor role for the hypotensive effect. Anyhow this study clearly indicates that alprenolol is valuable as an antihypertensive agent in combination with saluretics.

The significant decrease in blood volume from the 18th to the 30th week during continuous chlorthalidone treatment in this study agrees well with the results of Tarazi et al. (15). They used thiazide diuretics for periods from 6 to 24 months and demonstrated a persistent decrease in plasma volume. This gradual decrease in blood volume during chlorthalidone treatment in this study was not influenced by alprenolol. However alprenolol treatment changed the "normal" inverse relation between blood volume and PRA into a direct relation. The cause of this is obscure, but it is tempting to suggest the following hypothesis. Beta-

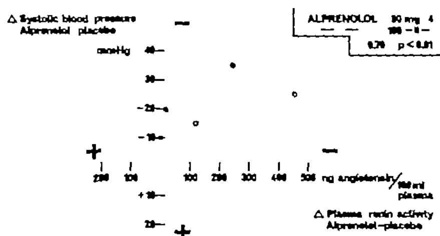


Fig. 1. Correlation between alprenolol-induced changes in PRA and BP.

Table X. *Effect of chlorthalidone and alprenolol on BP PRA and blood volume*

Parameter		A Without treatment ($\bar{X} \pm$ S.E.)	Diff. A–B (p)	B Chlorthalidone ($\bar{X} \pm$ S.E.)	Diff. B–C (p)	C Chlorthalidone + alprenolol ($\bar{X} \pm$ S.E.)	Diff. A–C (p)
Systolic BP	6	185 ± 7	< 0.10	162 ± 9	< 0.10	141 ± 6	< 0.05
Diastolic BP	6	115 ± 5		106 ± 3		94 ± 2	< 0.05
Supine PRA	5	96 ± 31	< 0.20	208 ± 62	—	66 ± 23	—
Erect PRA	5	80 ± 23	< 0.10	348 ± 102		128 ± 23	—
Blood volume	5	6.2 ± 0.7	—	5.7 ± 0.4		5.6 ± 0.5	—
Blood volume	5	6.2 ± 0.6[a]					

[a] Predicted values; see Methods.

adrenergic receptors are involved in a nervous mechanism for regulation of renin release in response to blood volume variations. Suppression of this mechanism by β-adrenergic blockade seems to expose an antagonistic active mechanism, which may be the sodium-sensitive macula densa mechanism in the kidneys, as earlier described (16). Normally this intrarenal mechanism is over ruled by the nervous mechanism.

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Acta med. scand. Vol. 193 pp. 197–200, 1973

A V NODAL RHYTHM IN ACUTE MYOCARDIAL INFARCTION

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Abstract. A-V nodal rhythm (NR) has been observed on monitoring during the first 24 hours in 79 (18%) of consecutive series of 450 admissions to coronary care unit (CCU) subsequently diagnosed as having an acute myocardial infarction. The concomitant CCU mortality as 23% as compared to 8% of the re mainder. Similarly the hospital mortality of 33% significantly differs from 19% in the remainder. The survivors were younger more rarely showed signs of power failure, and history of previous myocardial infarction was less common In 7 of the 26 patients who died, NR ppeared for the first and only time immediately prior to death. Survival of period of NR by more than one hour was not associated with raised mortality Distinguishing features for patients with NR were lower rate of previous myocardial infarction, higher incidence of larger infarctions as judged by SGOT maximum levels and also of inferior infarcts. The validity of the finding of NR in routine monitoring has been assessed by comparing ith continuous ECG recording in 95 consecutive patients. Of the 23 (24%) patients with NR on the ECG recording, the arrhythmia was observed in 14 and in further 4 cases where NR was claimed it could not be verified.

The improved knowledge of rhythm disturbances complicating the early phases of an acute myocardial infarction (AMI) has directed interest to those dysrhythmias which are associated with an increased mortality In a recent study from our coronary care unit (CCU) (1) a raised mortality was found to be associated with two supra entricular arrhythmias, atrial fibrillation and nodal rhythm (NR). Our findings regarding atrial fibrillation have been reported (2), and the present study concerns NR, which according to several authors is a benign arrhythmia (3, 4 7). Furthermore, in an attempt to evaluate the efficiency of monitoring for this often brief ar rhythmia a comparison with the results from continuous ECG recordings has been performed.

METHODS

Serafimerlasarettet serves an undefined population ithin Greater Stockholm and has about 200 beds for general medicine. The general policy of this CCU and the criteria used hav been previously described (8).

In the present study an analysis of NR during the first 24 hours was performed, as this is the period of care common to all patients. Prolonged CCU care was given only to patients ith certain complications, resulting in selection.

Policy of treatment NR as treated with digitalis withdrawal and atropine sulphate (0.5–1.0 mg) or methyl scopolamine (0.13–0.5 mg) intravenously depending on the ventricular rates.

Diagnostic criteria. The orking criteria in the CCU for the diagnosis of NR ere narrow (< 0.12 sec) and regular QRS complexes not activated by atrial P waves. Patients with A-V dissociation defined in the present context as nodal rhythm faster than, and interfering with, sinus rhythm, are included under the heading of NR in this study Furthermore NR, even when occurring agonally has been included in the study

Diagnostic reliability and ability The ECG of the patients was monitored from single bipolar chest lead and displayed on an oscilloscope. During 4 months continuous ECG was recorded with an 8-channel ink-jet writing electrocardiograph with paper speed of 10 mm sec (Mingograph 81 Elema-Schonander Solna, Sweden). The analysis of these recordings was compared with those obtained from routine monitoring (8)

MATERIAL

During the period studied (Jan. 1 1968 Dec. 31, 1969) there were 1 099 admissions to the CCU and in 450 (41%) diagnosis of AMI was made. Ther ere 284 men (63%), mean age 63 years, and 166 (37%) women, mean age 71 years. Forty-seven patients (10%) died in the CCU and further 48 (11%) died during after-care, giving total hospital mortality of 21%. The mean CCU stay lasted for 53 hours and the duration of hospitalization 21 days. Forty per cent of the patients were admitted within 3 hours of onset of ymptoms, 62% within 6 and 74% ithin 12 hours. ECG signs of AMI

Table I. *SGOT maximum levels and mortality for patients with and without NR during the first 24 hours in the CCU*

	With NR				Without NR				
	All patients		Mortality		All patients		Mortality		
SGOT max.	(N)	(%)	(N)	(%)	(N)	(%)	(N)	(%)	Mortality difference
1–99	13	16	2	15	157	42	15	10	NS
100–199	32	41	6	19	108	29	4	4	$p<0.05$
200–299	15	19	4	27	45	12	8	18	NS
> 300	11	14	6	55	36	10	17	47	NS
Not obtained[a]	8	10	8	100	25	7	25	100	NS
Total	79	100	26	33	371	100	69	19	$p<0.01$

Died prior to calculated max. enzyme rise.

were seen in 60% of patients and in 98% diagnostic enzyme rise was seen.

RESULTS

NR was seen as follows in the 450 patients:

Present on admission 10 (2%), observed during first 24 hours 79 (18%).

The 79 patients with NR during the first 24 hours in the CCU had a mean age of 68 years. Forty-two were men with mean age 65 years (range 55–87), and 37 women with mean age 71 years (range 55–87). The male/female ratio of 1.14 does not significantly differ from the remainder of the patients with AMI.

Mortality. The CCU mortality in the group with NR was 23% (18/79) as compared to 8% (29/371) in the remaining patients ($p<0.001$). Accordingly the total hospital mortality of 33% (26/79) was significantly higher when compared to that of the remaining patients, which was 19% (69/371) ($p<0.01$). NR did not, on the other hand, affect the mortality during the after-care period per se.

In 7 of the 26 patients who died, NR was diagnosed for the first and only time immediately prior to death. In 2 of these 7 cases NR was part of the bradyarrhythmias associated with rupture of the free left ventricular wall with tamponade, and this was probably also the case in a third patient on whom autopsy was refused. In one patient NR was first seen after repeated defibrillations. In two cases with shock and in one with frank pulmonary oedema, NR developed immediately prior to final asystole.

If these 7 patients with only agonal NR are omitted from the present evaluation, the CCU mortality for patients with NR is 15% (11/72) and the total hospital mortality 26% (19/72). Neither of these mortality figures differs significantly from that of the remaining patients.

When comparing hospital survivors with those who died, the former were found to be significantly younger: mean age 66 years, S.D. 10.8, as compared to those who died, mean age 71 years, S.D. 7.0 ($p<0.05$).

Past history. A history of heart failure was given by 29% (23/79) of the patients with NR and 21 (27%) were on digitalis at the time of admission. Neither figure differs significantly from that of the remaining patients. A history of previous myocardial infarction was significantly less common among patients with NR (14%: 11/79) than in the remainder (36%: $p<0.001$). Only one of the 11 patients with previous infarction was among the 53 survivors, whereas the other 10 died in hospital ($p<0.001$). No significant difference was found as regards angina pectoris; 56% of the patients with NR gave a history of angina pectoris as compared to 65% of the remainder.

Delay. The delay between onset of symptoms and admission was known in 71 of the 79 patients. Of those with a known delay 50 (70%) had arrived during the first 6 hours, which does not differ from the 67% amongst the remainder. The mean delay was 7.9 hours.

Enzymes. The maximum SGOT values and the mortality are given in Table I. In patients with

NR, infarcts with maximum SGOT values ≥ 100 units occurred in 82% as compared to 55% of those without NR ($p < 0.01$).

ECG site of infarction. The findings are given in Table II. It is seen that the patients with NR differ from the remaining patients with ECG signs compatible with AMI in a higher incidence of inferior infarctions. No differences as regards site of the infarction were found between survivors and those who died. Uncertain ECG findings were less common in patients with NR as compared to the remainder.

Power failure. Among the 79 patients with NR, 21 (27%) did not show signs of left heart failure during the first 24 hours and 5 (24%) of these died. This does not significantly differ from the mortality of 9% among the remaining 151 patients without failure. Of the 58 patients with failure and NR 6 had frank pulmonary oedema. Neither the incidence of left heart failure nor the respective mortality figures differ from the remainder.

NR was associated with hypotension without shock in 11 of the 79 patients, of whom 5 died. Clinical signs of shock in patients with NR were encountered in 10 (13%), all of whom died. Neither of these findings significantly differs from the remainder.

Combining all types of power failure, i.e. left heart failure, hypotension and shock, one or more of these was seen in 73% of those who survived hospitalization, in contrast to 96% of those who died ($p < 0.05$).

Temporal pattern. NR throughout the first day and continuing into the next day was seen in one survivor. In 25 cases there were repeated bouts of NR, 5 of whom died. In 53 patients there was only one episode of NR and 21 died.

Ventricular rates and therapy. Owing to the shortness of many of the periods of NR no accurate account of the ventricular rates can be given. Atropine or methyl scopolamine was given to 31 patients, of whom 12 had associated hypotension or clinical shock, the indication being absolute or relative bradycardia in the remainder. Treatment with pacemaker was applied in one patient.

Validity. During a period encompassing 95 consecutive patients with verified AMI, routine monitoring diagnosed NR in 18 (17%) patients. Of these the diagnosis was verified from the continuous ECG recordings in 14 and rejected in 4. Altogether 23 patients (24%) were found to ha e NR at the analysis of the ECG recordings. False positive findings therefore amount to 4/14 (22%) and false negative to 9/23 (39%). Continuous ECG recordings, furthermore, revealed NR mainly as an escape mechanism in 20 and as an active capture rhythm in 3 of 23 patients.

Table II. *Infarction site according to ECG for 79 patients with NR complicating AMI as compared to 371 without NR during the first 24 hours in the CCU*

ECG site of infarction	With NR N	%	Without NR N	%	Significance
Uncertain[a]	19	24	161	43	$p < 0.01$
Anterior	20	25	90	24	NS
Anterolateral	11	14	23	6	NS
Inferior	21	27	42	11	$p < 0.05$
Inferolateral	5	6	32	9	NS
Lateral	1	1	13	4	NS
Combined[b]	1	1	5	1	NS
Anteroinferior	1	1	5	1	NS
Total	79	100	371	100	

Includes BBB and subendocardial infarction.
Indicates ECG changes over anterior, inferior and lateral walls.

DISCUSSION

In the present investigation concerning NR in the early phases of an AMI it was found that this dysrhythmia is associated with a significantly raised mortality. In previous investigations of arrhythmias complicating AMI it has commonly been accepted that arrhythmias occurring terminally should not be included, nor those observed immediately after treatment of ventricular fibrillation (3). In the present study all instances of NR have been included, resulting in a poor prognosis. If those instances of NR, referred to as terminal according to the above criteria, are excluded, a significantly raised mortality remains—19% for the CCU stay and 29% for the total hospital stay. If on the other hand, the 3 instances where NR was part of the bradyarrhythmic complex associated with rupture of the left ventricular wall and tamponade are also classed under the heading of terminal arrhythmia and therefore excluded, then the arrhyt loses its prognostic importance. This fin

in accordance with several previous reports (3, 4, 7). From a practical viewpoint it may be noted that, *if a patient survives a period of NR by* more than one hour, the prognosis seems unaffected.

Further analysis revealed that, for unknown reasons, previous infarction was less common in patients with NR. Smaller infarcts as estimated by maximum enzyme levels seem rarer, as are also "uncertain" ECG findings, when compared to the rest of the patients in the present material. Inferior infarcts significantly predominate among cases with NR, which has been reported previously by other authors (4, 10).

When comparing patients with NR who died in hospital with those who survived, the former were found to be older, more often to have a history of a previous infarction, as well as showing signs of power failure. There were no similar distinguishing features when comparing the agonal NR patients with those with NR and who died later during hospitalization.

The incidence of NR in the present study is comparatively high (18%) when compared to the 2–10% in other series (3, 4, 5, 7). This high incidence is in part explained by the inclusion of the patients with terminal NR. Yet, even without these patients, the incidence remains high. The continuous ECG recordings showed that NR in fact occurred in 24% of 95 consecutive patients with AMI. The finding that false negative diagnoses are as common as 39% accords with findings on the diagnostic accuracy of monitoring in short bouts of ventricular tachycardias in a similar study on the same patient material (8).

The absence of any overrepresentation of patients with power failure among those with NR is compatible with the unaltered prognosis when patients with agonal NR are excluded. The present authors still feel reluctant to discard the diagnostic importance of this arrhythmia, as it may be an early warning of rupture of the free left ventricular wall and tamponade (9) which in the future may become a reversible condition (6).

ACKNOWLEDGEMENT

This study was supported by grants from the Swedish National Association against Heart and Chest Diseases.

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Acta med. scand. Vol. 193 pp. 201–205 1973

LOSS OF CONSCIOUSNESS FROM ARRHYTHMIA. THE PATIENT'S EXPERIENCE

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Abstract. With improved treatment of circulatory arrest our knowledge of patients' experiences from such situations has increased. This report is based upon interview with patients treated in CCU or stimulated with an artificial pacemaker. As reported earlier, the patients suffering from arrhythmic syncopes felt no anxiety at the time of the attacks. The attitude of the physician after the resuscitation and the information given to the patient seem to be of great importance for the patient' attitude afterwards. A penetrating report is given of the reactions of one patient with 50 attacks of entricular fibrillation to the ECG monitoring and the attitude of the staff in the CCU. Examples of reactions of relatives of resuscitated patients with resulting cerebral death and of relatives of desperately ill pacemaker-treated patients are also briefly reported.

Our opportunities of recording the experiences of persons losing consciousness as a result of ar rhythmia have greatly increased as a result of the advances in the treatment of circulatory arrest during the last decade. At the time of the attack the person is usually unaware of the life-threatening situation, and the experience of possibly imminent death will therefore not ha e been preceded by a systematic analysis of the realization that there will come a time when he no longer exists. This is, of course, quite a dif ferent experience from that of dying, with the fight against death.

The views presented here are based on the reports of patients at Serafimerlasarettet who ha e suffered from circulatory arrest and either been provided with a pacemaker and then followed up, or else admitted to the Coronary Care Unit (CCU) at this hospital.

THE PATIENT'S EXPERIENCE

While the state of unconsciousness produced by arrhythmia associated with asystole or entricular fibrillation is usually described as having a sudden onset, this is by no means always the case' there may be premonitory symptoms, produced perhaps by extreme bradycardia or ventricular tachycardia preceding the circulatory arrest.

As it is usually described, the experience includes irregular heart action a wa e of warmth, passing up towards the head, buzzing in the ears, a feeling that the pulse is weakening; a mist before the eyes, accompanied by an agreeable feeling and a wish to lie down and rest, a sensation of an enveloping numbness, which cannot be described. At the onset of these warning signs patients with recurrent episodes of arrhythmic syncope (Adams-Stokes attacks) usually lie down or otherwise try to eliminate the risk of hurting themselves, to be relieved of the fear of injury was regarded as the greatest advantage of a pacemaker by 60% of the pacemaker patients in an interview study 17% mentioned the life-sustaining effect as the most highly esteemed benefit (6). During the year prior to receiving the pacemaker two of the patients in that interview study had been almost constantly confined to bed because either the patient or the relatives were afraid of his falling and injuring himself

Other patients in the same series who, despite attacks of arrhythmic syncope, felt no anxiety or apprehension before receiving the pacemaker did so after failing pacemaker function. An example is provided by the following case.

Case 1. The first pacemaker unit supplied to a woman aged 60 years because of few attacks of arrhythmic syncope developed failure immediately after it had been implanted. Afraid that the second unit would also fail, the patient dared not go out during the first 18 months. During the first 2 months she would phone several times eck to express her intense fear of

another breakdown. When she ultimately came to realize that, if she could have confidence in the physician, she could be rehabilitated completely her extremely apprehensive attitude disappeared.

That a coronary patient acquainted with the risks associated with the disease does not necessarily become more anxious after circulatory arrest is evident from the following report.

Case 2 A man, aged 63 years, who had had two myocardial infarctions was admitted to the Emergency Department in an unconscious state. Heart massage was given, manual ventilation was introduced and after about 10 min a regular sinus rhythm and good circulation were noted. A rapid infusion of alkali was given and ventricular fibrillation was stopped by defibrillation, about 1 min after the sinus rhythm had been recovered he regained consciousness. He related in detail the situation when he first began to feel ill. He complained of pain at the site in the chest where there was marked tenderness, probably owing to a costal fissure caused by the heart massage. He had no fear nor did he express any apprehension that circulatory arrest might recur. Before suddenly fainting he had not had any discomfort, apart from a mild sensation of arrhythmia. Even when one of us contacted the patient a day later there was no evidence that he was concerned about the circulatory arrest.

The anxiety that some patients display and sometimes talk about in connection with pulmonary oedema was not observed in any of the patients we talked with after arrhythmic syncope. An example is provided by the following patient with both these conditions.

Case 3 A man, aged 63 years, was admitted to the CCU with evidence of a major infarction. After about 1/2 hour ventricular tachycardia developed, continued for about 30 sec and passed spontaneously. He lost consciousness but soon woke up and continued talking. He displayed no signs of anxiety and accepted the explanation that the heart had stopped for a moment and he had fainted. He expressed no disquiet at this. During the next 2 hours increasing cardiac incompensation gradually developed, and, despite intensive treatment, led to frank pulmonary oedema. In the 15 min prior to this, in spite of sedation, he had been greatly distressed, manifested as motoric anxiety and an attitude as of seeking help. The oedema could not be relieved, cerebral activity gradually diminished and the patient was unconscious for more than 5 min before the respiration and circulation finally stopped. Autopsy disclosed a recent myocardial infarction of the greater part of the left ventricle.

The paralysing fear and anxiety previously felt by some patients with circulatory arrest in connection with classic myocardial infarction accompanied by pain were not mentioned by any of the patients in their account of their experience before or after ventricular standstill or ventricular fibrillation.

An account by one patient includes an exhaustive description of his impressions not only of the loss of consciousness but also of his reactions to the oscilloscopic monitoring and the attitude of the staff. It is highly informative and merits report in full.

Case 4 A man, aged 40 years, was admitted to hospital after fainting at work. Apart from concussion when 20 years old, with fairly frequent headaches, and a number of minor operations, the patient had previously been in essentially good health. On a few occasions during a month or so before the present illness he had felt pressure over the chest that was not related to effort.

He was found lying over his desk at work, and efforts during 5–6 min to waken him were of no avail. There were no convulsions or passage of urine or faeces, nor had he had any warning signs of the attack.

On admission to hospital he was pale, without evidence of heart failure, and oriented in time and space. Over the precordium a regular rhythm of 80 beats/min was recorded with normal heart sounds and no extra sounds or murmurs. A routine neurological examination disclosed no abnormal conditions. Constant oscilloscopic monitoring was introduced. About 6 hours after admission there were short episodes of ventricular tachycardia, which started after VEB in the form of R on T. The ventricular arrhythmia was stopped by infusion of lignocaine. During the first day the ECG showed intermittent antero-lateral BBB and slight S-T and, especially, T changes. There was no evidence of a demarcation potential. Repeated transaminase determinations and routine laboratory tests were normal. The ESR on admission was 10 mm/h and subsequently rose to 35.

Because of slight right-sided ptosis and suspected pathological nystagmus during lateral gaze a further neurological examination was carried out. An EEG showed a minor episodic anomaly in the left fronto-temporal region. The cerebrospinal fluid was normal. Nor did encephalography disclose any remarkable features apart from slightly wider lateral ventricles on the left than the right side. At discharge a tentative diagnosis of myocarditis was made.

About one year after the first fainting attack the patient had another such attack, on this occasion passing urine. Immediately after admission ventricular fibrillation was recorded, and defibrillation was performed, but it recurred some 50 times in the next 24 hours and could not be completely eliminated by various drugs. On this occasion, too, no diagnosis of infarction could be made. A few insignificant increases in the enzyme activity were recorded, and these were ascribed to the frequent defibrillation procedure. Because there was thought to be a link between the mental symptoms and the ventricular fibrillation, it was considered possible that this might have been triggered by an increased secretion of catecholamine, with effect on the myocardium.

After long period of supervision at the Coronary Care Unit, during hich there was no new episode of arrhythmia, and after convalescence in the hospital with mobilization and bicycle exercise, the patient was discharged in the eighth week with only minor symptoms.

THE PATIENT'S EXPERIENCE OF THE PERIOD OF ILLNESS

About one year after discharge the patient was asked a number of questions relating to his personal impressions of the illness. He gave the following overall reply:

My general recollection of the emergency period during hich there as repeated loss of consciousness is calm and harmonious state of mind, hich surprises me. I recall no feeling of alarm although I was consciously aware of the seriousness of the situation. In some way I was certain in my mind of happy outcome.

Question 1 Did you have any prior arning signs of disturbed heart rhythm? If so, what?

Reply The attacks of unconsciousness at my place of work came without warning. Nor did I hav warning of the first episode of arrhythmia at the hospital when I was admitted for the second time, but in some way I was aware that the situation this time was different. Moreover, the optimistic explanation given me on the first occasion now seemed unrealistic. The repetitions made me more alert, and I began to fear them. I do not recall if there were any warning symptoms but I do know that I tried as long as possible to resist falling asleep during the arrhythmia episodes. I remember this as spontaneous, intensive fight to retain consciousness, perhaps feeling that I was fighting for my life; I still have vid memory of this fight although it cannot have lasted more than "a moment". It began when I sensed the onset of the arrhythmia. I remember feeling of giddiness, swaying as if dropping off to sleep, condensed version of falling asleep under an anaesthetic. There was often feeling of tension developing in the chest, but no particular movements in the region of the heart or other phenomena that I ould ascribe to the heart action. I also recall the onset of cold sweat.

Question 2 What do you remember to have experienced on waking up again?

Reply I have practically no recollection of waking up, I as suddenly there lying awake. I could resume conversation almost immediately. The first thing I remember of the treatment (defibrillation) was of the chest being cleaned after the electrodes had been removed. I do not recall any transitional stage between unconsciousness and being awake; I recall no pain, discomfort or anything else after waking, only natural tiredness. Nor have I any memory of retrospective phenomena or of thinking ahead. Life went on just as it did before I fell asleep.

Question 3 What observations did you make regarding the staff reactions after the episodes of arrhythmia?

Reply I do not recall always seeing staff in the room when I woke up; I pondered on this when in hospital and as somewhat surprised at it, but supposed that I was being watched from the monitor room and that the nurses did not wish to disturb me unnecessarily. This perhaps shov that in fact some time elapsed after the arrhythmia before I was fully conscious and that the staff had left in the meantime. At no time did I notice any particular reaction of the staff; they just seemed to be engaged in their normal round of duties. Between the episodes of arrhythmia there was, of course, some discussion of my condition by the medical personnel, from which I got an impression of uncertainty and perhaps also of uneasiness among the doctors. The other members of the staff appeared to be going about their work in an efficient way and not giving special attention to my case. I recall no particular reaction in connection with the individual arrhythmic episodes.

Question 4 Were you concerned about recurrence of the arrhythmia during the time you ere in hospital or after discharge?

Reply. A day or so after the last attack of unconsciousness I began to feel relatively calm and, of course, more so as time ent on and there was no recurrence. As long as I was still in the emergency room under constant supervision the sense of security increased, but when I was moved to ward the fear of recurrence returned. With each change involving withdrawal of supervisory measures my apprehension grew—for instance, on being moved to the ward, removal of the monitoring unit there during the day switching off the apparatus also at nights, during the first walk outside the room, etc. Particularly at nights after the monitoring unit had been switched off I often felt great anxiety even though I had been given sedatives. I was extremely concerned with my heart activity I tried to master this feeling of anxiety by intellectual effort; on only one occasion did I fail, and had to call the night nurse, tell her of my apprehension and ask her to examine me and switch on the monitor. Before discharge my disquiet naturally increased again. After my first days away from hospital I returned with feeling of relief. The fear of new episodes of arrhythmia were intensified after I had been discharged. My wife and I adapted our way of life so that for long period after my discharge I always had her or some other capable person near at hand. When I was in the company of other people my anxiety about the arrhythmia diminished, hereas when I was alone, especially at nights, it remained for quite long time. It is difficult to say just hen it disappeared. There are still occasional nights when I wake up after troublesome dream with the feeling of anxiety hich compels me to get up and take my medicines. Sometimes I have an uncomfortable feeling that the dream was premonition of arrhythmia or even result of weak arrhythmia.

Question 5 Do you believ that your feelings would ha been different if the staff had adopted another attitude?

Reply I believe that the cool efficient attitude and approach in managing the problem during the emergency period were the correct ones in my case. The impression I had of uncertainty and anxiety among the doctors—

mentioned under question 3—was not stronger than was needed to give me feeling of security I am personally most critical of people who display confidence and optimism that is inconsistent with my assessment of the situation. This applies to the emergency period, but this was over I felt the need for complete and unconditional information on the situation, the possible implications and various hypotheses. I prefer an admission of ignorance to taciturnity I feel for my part that there is, in principle, much in the view that the treatment of patient in more complicated cases should be managed as teamwork, with the patient as one of the team rather than more or less well informed object.

REACTIONS OF RELATIVES

The ethical (2) and medicolegal (3) aspects of re suscitation have become increasingly relevant in recent years in connection with the rapid advances in transplantation surgery On the other hand, the sometimes intense mental stress to which the relatives are exposed has received little attention.

The relatives of a patient who has been resuscitated with resulting cerebral death sometimes feel no reason to make contact with the patient because they cannot bear a situation changing between hope—even if false—and hopelessness.

In other cases the relatives may see the treat ment as futile and perhaps even degrading for the patient.

Two examples will be given of situations where the relatives of pacemaker patients expressed trong apprehension that the treatment would prevent a natural death.

Case 5 A man, aged 91 years, who had used pacemaker for the previous 4 years because of arrhythmic syncope, developed cerebral confusion and had few clear moments H also suffered from prostatic car cinoma. The relatives wished to have the pacemaker switched off, since they believed that this prevented natural death. Only after long and detailed explanations could they be convinced that the patient would die in due time despite his pacemaker

Case 6 Over period of 2 months a man, aged 88 years, ho had been using pacemaker for the previous 8 years had increasing cerebral confusion owing to arteriosclerosis. His ife, ho could not bear to see her husband in this state, asked that the pacemaker should be switched off. She could not be persuaded that her husband would probably not live much longer because of severe heart failure. The wife state of mind was such that she could not be made to change her mind, despite good relationship developed over the years after meeting one of us at checks of her husband's pacemaker unit. Her demand as, of course, not acceded to. The patient died some days after this conversation.

DISCUSSION

The impression obtained here that patients with circulatory arrest do not have a very strong ex perience of it has been reported earlier On the subject of resuscitated patients who had had circulatory arrest Ask-Upmark (1) writes: "I have asked the patients if they remembered anything about the visit to the other side and invariably hitherto, they have denied remembering anything at all. Burch et al. (4) summarize their ex perience of interviews with resuscitated patients thus: Biologic death is not an unpleasant experience. To man it is only a deep eternal sleep.

A number of pacemaker patients, who had fainting attacks due to arrhythmia before the pacemaker system was provided, seemed to have adopted the attitude that their life was essentially finished, some appeared not to accept that the pacemaker can actually prolong their life. Several months may pass before they recognize their new situation. Here it is thus not the fear of actually dying to which the patient has altered his attitude rather he has tried to push out of his mind an earlier acceptance of the fact that he will cease to exist. Paradoxically enough, the idea that the pacemaker will prolong life has, for some, had a paralyzing and for others a disturbing effect on their emotional life.

The level of anxiety for patients treated in a CCU has recently been analysed and shown not to be related to the physical severity of the patient's state (5). Very few patients in that study felt a dependence on the machines and, when this occurred, it was a transient phenomenon. In one case reported here the patient felt anxiety when supervision was terminated. It is of importance to know the patient's reactions to the treatment in a CCU with its often abrupt changes in the degree of supervision, to be able to give the necessary support.

The approach of the attending physician to resuscitation is certainly a major factor deter mining the patient's own attitude to circulatory arrest. If this event is described as, for example fainting due to irregular heart action or running of the heart or a short interruption of the heart action, many patients will probably not attach more importance to the circulatory arrest than to, for instance, vasovagal fainting ex perienced by many people.

ACKNOWLEDGEMENTS

The study was supported by grants from the Swedish National Association against Heart and Chest Diseases and the Martha and Gunnar Gordon Foundation.

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Acta med. scand. Vol. 193 pp. 207–210, 1973

THE SIGNIFICANCE OF PSYCHOLOGICAL EVENTS IN A CORONARY CARE UNIT

PRELIMINARY REPORT

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Abstract. Thirty-six consecutively admitted patients of both sexes aged 45–83 years have been interviewed during their stay at the coronary care unit at the Serafimerlasarettet. The interviewers were not aware of the patients' clinical diagnoses nor their frequency of ventricular arrhythmias. Information was collected concerning number of visits by relatives and kind and number of disturbing items at the ward during the 24-hour period which followed the initial 8–18 hours after admission. This information was analysed in relation to the kind and frequency of ventricular arrhythmias. The number of disturbing items was similarly distributed in the two diagnostic groups (definite or suspect myocardial infarction, $N = 17$ and observation cases, $N = 19$). The number of relatives' visits was higher in the infarction group during the studied period ($p < 0.01$). In both diagnostic groups, which were similar with regard to age and sex, a tendency was observed for patients with potentially more serious arrhythmias to have more visits by relatives. This could not be explained by the relatives' knowledge of the medical condition of the patient. A tendency was also observed for patients in both groups with more ventricular arrhythmias to report more disturbing items not related to their coronary care at the ward. The findings are discussed against the background of findings of levels of free fatty acids in the blood, which were elevated in both diagnostic groups (to a similar extent), and of known effects of catecholamines in stressful conditions.

A growing literature on catecholamines in relation to arrhythmias shows that an increased output of epinephrine and norepinephrine increases the frequency of potentially life-threatening cardiac arrhythmias (9, 13). Especially the development of ventricular arrhythmias seems to be facilitated by catecholamines and, particularly so during the course of a myocardial infarction (5, 6, 7). Studies in a coronary care unit (CCU) also verify that anxious myocardial infarction patients develop more arrhythmias than do others (12).

A study was performed on cats with experimentally induced myocardial infarction whose hearts were cut free of sympathetic and parasympathetic nerves, compared to cats with similarly infarcted but nerve-intact hearts. This experiment demonstrated that the former group of cats exhibited strikingly less ventricular arrhythmias than did the latter group (1). This seems *to indicate that emotions*—both "positive" and "negative"—which induce increased sympathetic and/or parasympathetic nerve activity could play a significant role in the development of ventricular arrhythmias. The levels of catecholamines are known to be "emotion-sensitive" (8, 11).

Not many studies have been performed on arrhythmias in relation to psychological events in CCUs. However, such a study by Thurman and Bruhn (12) seems to verify that psychologically important events in the unit have a significance in the development of arrhythmias during the course of a myocardial infarction.

The present study was a similar effort to study psychologically important events in the CCU in relation to arrhythmias.

MATERIAL AND METHODS

Thirty-six consecutively admitted patients at the CCU at the Serafimerlasarettet were subjected to the study. The age and sex distribution in relation to diagnosis is seen in Table I. The criteria for admission to the CCU and for the diagnosis of acute myocardial infarction have been published elsewhere (10). All patients of both sexes filling the criteria for admission were included in the study, the only exception being four patients who were in a seriously deteriorated general condition, making 10-min interviews impossible.

A short *questionnaire was used, including simple* questions about visits (mostly spouses or children), worries because of statements from personnel, noise from the ward, awareness of emergencies in other patients and

Table I. *Age and sex in relation to diagnosis*

	Definite or suspect myocardial infarction		Observation cases		Total	
	Males	Females	Males	Females	Males	Females
N	14	3	10	9	24	12
Age (y)						
Range	48–74	63–82	45–67	52–83	45–74	52–83
Mean	63	74	59	67	61	69

painful or otherwise worrying examinations or operations. The interviewer was not aware of the patient diagnosis, e.g. whether he/she had turned out to have positive diagnosis of myocardial infarction or not, and did not know the patient possible history of arrhythmias at the ward. The interviews were made as soon as the patient condition allowed, mostly 8–18 hours after admission to the ward. All events that might have taken place during 24 hours after the admission period—the latter including about 2 hours after the patient's entrance at the CCU—were explored.

The schedule of events was mostly recorded on two occasions, one in the middle and one at the end of the 24-hour period.

All arrhythmias were continuously monitored, recorded and coded during the 24-hour period. In the present study the interest was focused on the following arrhythmias which were recorded as ventricular:

Ventricular fibrillation, ventricular tachycardia, mono- or multifocal ventricular premature beats with frequency of at least 1/min, multifocal ventricular premature beats with lower frequency than 1/min.

Blood samples for the analysis of plasma free fatty acids (FFA) were drawn at fixed times of the day and night (8 a.m., 11 a.m., 4 p.m. and 11 p.m.) before meals. The plasma content of FFA was analysed according to Hagenfeldt (3). Events reported to have occurred during the last hour before the blood sample was drawn were recorded separately.

The CCU at the Serafimerlasarettet has been described elsewhere (4). It is a closed unit with 7 isolated rooms. In the central room the nurses are able to observe the patients through windows.

Operations caused by arrhythmias or other complica-

Table II. *Number of visited patients in relation to arrhythmias during the same 24-hour period*

	Definite or suspect myocardial infarction		Observation cases	
	Arrhythmia	No arrhythmia	Arrhythmia	No arrhythmia
Visit	12	3	4	3
No visit	1	1	2	10

Table III. *Mean number of visits in relation to kind of ventricular arrhythmias during the 24-hour period*

	Definite or suspect myocardial infarction		Observation cases	
	Mean no. of visits	No. of cases	Mean no. of visits	No. of cases
Ventricular tachycardia	1.75	4	1.67	3
Multifocal or monofocal ventricular premature beats ≥5/min	1.43	7		
Monofocal ventricular premature beats <5/min	1.50	2	0.33	3
No arrhythmia	1.00	4	0.23	13

tions of the patients' disease were not registered as "disturbing items". Only operations which were routinely performed in all cases (such as blood sample drawing at regular intervals), but considered painful or distressing by the particular subject, were registered as disturbing items.

RESULTS

The distribution of ventricular arrhythmias (see Material and methods) during the 24-hour observation period in relation to visits (by relatives or friends) during the same period is shown in Table II. Of the patients with definite and with suspected acute myocardial infarction 15 had had visits and only 2 had not, while 7 of the observation cases had had visits and 12 had not. The difference between the mean number of visits in the infarction group and in the observation group is significant ($p < 0.01$).

Arrhythmias occurred more often in the patients with definite and with suspected myocardial infarction than in the observation cases. In the infarction group 12 of the visited 15 patients and 1 of the 2 non-visited patients had arrhythmias. In the observation group 4 of the 7 visited patients and only 2 of the 12 non-visited patients had had arrhythmias. Thus, it seems that arrhythmias occurred more frequently in patients who had had visits.

In Table III the mean number of visits has been calculated for patients who had had ventricular tachycardia, multifocal ventricular premature beats or monofocal ventricular premature beats ≥5/min, monofocal ventricular premature

Table IV *Number of patients with "disturbing items" in relation to arrhythmias*

	Definite or suspect myocardial infarction		Observation cases	
	Arrhythmia	No arrhythmia	Arrhythmia	No arrhythmia
Disturbing items	6	1	3	2
No disturbing items	7	3	3	11

beats 1–5/min, and for patients who had not had any ventricular arrhythmias. The mean number of visits was lowest for observation cases without arrhythmias and highest in the infarction group with ventricular tachycardia. Patients with high frequency of visits seem to have more severe arrhythmias.

The distribution of ventricular arrhythmias during the 24-hour observation period in relation to the presence of disturbing items during the same period is presented in Table IV. Twelve of the patients had experienced disturbances and 24 had not. In the infarction group 6 of the 7 patients with disturbances and 7 of the 10 patients without disturbances had had arrhythmias. In the observation group 3 of the 5 patients with disturbances and only 3 of the 14 patients without disturbances had had arrhythmias. Thus there seems to be a tendency to more arrhythmias in patients with disturbing items.

A compilation of the disturbing items reported is made in Table V. Noise in the ward and worries because of disease information were the most common disturbances, while conflicts with personnel and painful routine operations were experienced only by one subject in each diagnostic group.

In the observation group a larger number of the elderly subjects were "disturbed". In the infarction group no age trend was observed.

The material was not sufficiently large for the analysis of time relations. In several cases an increased number of ventricular premature beats was observed during the relatives' visits.

Compared to normal subjects, the levels of plasma FFA were raised in the patients with definite or suspected myocardial infarction as well as in the observation cases, although there was no significant difference between the infarction and observation groups (4). No statistically significant relations were observed between FFA levels and disturbing items or between FFA levels and visits.

DISCUSSION

The two diagnostic groups were comparable as to age and sex distribution. Despite this similarity the observation cases were visited much less frequently than the infarction cases. This difference may be explained by the fact that a diagnosis of infarction has been considered to be more serious than possible diagnoses in the observation group. This would increase the tendency of relatives to visit the infarction group. However most patients in the non-infarction group had coronary heart disease, and because of the delay of laboratory tests, etc. the distinction between myocardial infarction and "no myocardial infarction" in most cases could not be made during the 24-hour period that was studied. Hence the diagnosis as such could not have influenced the number of visits significantly. Thus the difference may reflect a psychosocial difference between the groups. This will be subjected to further analysis. The small size of the subgroups makes χ^2-tests non-meaningful. However the impression is

Table V *Number of patients who reported "disturbing items"*

	Definite or suspect myocardial infarction	Observation cases	Total
Noise in the ward	3	3	6
Worries because of disease information	2	3	5
Conflicts with personnel	1	1	2
Painful routine operations	1	1	2
Worried by radio listening	1	0	1
Total	8	8	16

gained that patients who are visited by relatives or friends have more ventricular arrhythmias than those who are not visited (Table II). This applies especially to the observation group, whereas in the infarction group most patients had ventricular arrhythmias and visits. When a subdivision of arrhythmias was made (Table III) it was observed that the number of visits was roughly proportional to the severity of ventricular arrhythmia in each diagnostic group. This could support the hypothesis that many visits of relatives may be dangerous for the myocardial infarction patient during the first days. On the other hand, the bias created by the tendency of nurses to call for relatives when the patient is *critically* ill has not been totally eliminated in the study. This may apply to patients with ventricular fibrillation. However ventricular fibrillation occurred in only one patient in the study.

Table IV shows that there is a tendency for non-arrhythmic patients, in both diagnostic groups, to be less disturbed by the stay in the CCU. Disturbing items caused by treatment of arrhythmias have not been included in the study. The non-arrhythmic patients less often complained of noise in the ward and were less often worried. Whether this reflects a causal relationship—anxiety increasing the risk of ventricular arrhythmias—or the fact that the experience of arrhythmias is frightening and provokes an increased catecholamine output is not apparent from the study.

It seems to us that the reported frequency of conflicts with personnel was relatively small. This is consistent with the impressions of Hackett et al. (2), who studied 50 consecutive myocardial infarction patients in a partially open and partially isolated CCU. None of their patients complained spontaneously about the atmosphere of the unit. Since medical personnel made the interviews both in our study and in the study of Hackett et al. this finding may simply reflect the patient's wish to please the unit personnel, but probably also indicates that the patients feel security while they are at the unit.

Another observation was that the patients in the observation group seemed to experience about as many disturbing items as did myocardial infarction patients (Table V). The analysis of FFA serum levels also showed that both groups had high FFA levels, not significantly higher in the infarction patients, which may indicate similar degrees of sympathetic hyperactivity in the two groups.

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish National Association against Heart and Chest Diseases and the Swedish Medical Research Council (no. 19X-3197).

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Acta med. scand. Vol. 193 pp. 211–214 1973

REPRODUCIBILITY OF THE ECG CLASSIFICATION SYSTEM OF THE MINNESOTA CODE IN THE STUDY OF PATIENTS WITH CORONARY HEART DISEASE

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Abstract. Electrocardiograms of 412 male postmyocardial infarction patients recorded 1–2 years after their first infarction have been classified according to the Minnesota Code by two independent readers (physicians). A study of the inter- and intra-observer error or reliability was then performed. The degree of accuracy and reproducibility thus obtained was related to degree of experience in the use of the Minnesota Code. Reproducibility was greater towards the end of the study when the readers were more experienced. The importance of substantial and continuous experience with the use of the Minnesota Code is stressed as well as the importance of at least two observers reading the ECGs independently. A final arbitration is no problem. These criteria must be fulfilled before the use of the Minnesota Code in clinical or epidemiological studies on coronary heart disease.

The ECG has proved a useful epidemiological survey method in large population groups. The accuracy of such screening was increased by the introduction of the standardized ECG classification system of the Minnesota Code (3).

This code makes possible a uniform and objective registration of clearly defined ECG changes without any attempt at primary interpretation.

We have applied this system in a follow-up study of survivors of a first myocardial infarction (9, 10) where coronary events were related to the original ECG changes. The validity of the results is of course dependent on the accuracy, reliability and reproducibility of the method employed. Many authors have considered this problem with reference to the Minnesota Code (2, 4, 5, 7, 12). Our study examines the inter- and intra-observer error in coding the ECGs of 412 survivors of a first myocardial infarction.

MATERIAL AND METHODS

The study deals with the ECGs of 412 survivors of first myocardial infarction previously described in the Oslo diet-heart study (8, 12).

A conventional 12-lead resting ECG including the standard leads I, II and III, the unipolar leads VR, VL and VF and V_{1-6} was recorded 1–2 years (mean 20 months) after the first myocardial infarction. Two of us (J. B. and I. H.) coded the ECGs according to the ECG classification system of the revised Minnesota Code (13). Each reader read independently and was ignorant with regard to coronary events during the follow-up period.

Inter-observer error was studied by systematically comparing the codes obtained by the two observers for each ECG. The correlation to degree of experience was studied by comparing the inter-observer reliability of the first 50 ECGs with that of the last 50 of 412 ECGs.

Intra-observer error (reproducibility) was studied by letting one observer code twice the same ECGs with some time lag. First and second codings are then compared and the degree of reproducibility studied. Again the results of the first 50 were compared with the last 50 of 412 ECGs.

RESULTS

Table I shows the degree of accuracy–inter-observer error when the observers are relatively inexperienced. The main criteria, Q, ST and T changes, show a relatively high degree of discrepancy. Twenty-five of 31 ECGs with Q changes were coded identically, whereas 5 of 19 with ST depression and only 14 of 33 with T changes received the same code numbers.

The same comparison for the last 50 of the 412 ECGs is also shown in the Table. The degree of inter-observer reliability has increased: 24 of 30 ECGs with Q changes, 9 of 15 with ST de-

Table I. *Inter-observer error*

Item	Main group code	Sub-group code	No. of sub-groups	Agreement between 2 observers (no. of tracings): Absent – Total discrepancy	Absent – Main group discrepancy	Absent – Subgroup discrepancy	Present	Total no. of items
First 50 ECG tracings								
Nothing reportable	0	—		3	—	—	5	8
Q and QS	1	1-1 to 3-6	21	6	4	2	25	31
Axis items	2	1 to 5	5	3			7	10
High R waves	3	1 to 3	3	2			2	4
ST depression	4	1 to 4	4	14	10	4	5	19
T wave items	5	1 to 4	4	19	11	8	14	33
A-V conduction	6	1 to 5	5	0			1	1
Ventricular conduction	7	1 to 6	6	2			3	5
Arrhythmias	8	1 to 9	9	1			2	3
Miscellaneous	9	0 to 8	8	8			26	34
Last 50 ECG tracings								
Nothing reportable	0	—	—	1			4	5
Q and QS	1	1-1 to 3-6	21	6	2	4	24	30
Axis items	2	1 to 5	3	1			6	7
High R waves	3	1 to 3	3	4			2	6
ST depression	4	1 to 4	4	6	2	4	9	15
T wave items	5	1 to 4	4	9	3	6	19	28
A-V conduction	6	1 to 5	5	1			0	1
Ventricular conduction	7	1 to 6	6	0			5	5
Arrhythmias	8	1 to 9	9	3			1	4
Miscellaneous	9	0 to 8	8	9			20	29

pression, and 19 of 28 with T changes are identically coded by the two observers.

Table II demonstrates the intra-observer reliability or reproducibility i.e. 100 ECGs coded twice by the same observer.

The first 50 of 412 ECGs: 16 of 30 ECGs with Q changes received the same code number twice, as did 12 of 23 with ST depression and 20 of 34 with T changes.

The last 50 of 412 ECGs: 26 of 31 ECGs with Q changes received the same code number twice. This was also the case for 6 of 15 with ST depression and 20 of 28 ECGs with T changes.

DISCUSSION

The introduction of an ECG classification system, the Minnesota Code (1), has provided a uniform and clearly defined system for recording ECG findings. The accuracy and reproducibility of the readings of the ECGs is a crucial point both in studies dealing with patients and larger population groups. This problem has engaged epidemiologists to a considerable extent (2, 4, 5, 7, 12) and was indeed partly responsible for the introduction and later modification of the Minnesota Code (13). In previous studies the degree of agreement between different interpreters is in the order of 75–90% depending on what ECG criterion is referred to (7). Thus there is a greater degree of agreement with regard to Q changes than with regard to J point or T wave amplitudes.

Elgrishi et al. (6) compared the reading according to the Minnesota Code of 80 ECGs by four specially trained technicians. They found the differences in interpretation between observers to be in the same order as those found for the same observer. They suggest a value of the coefficient of accuracy somewhere between 82% and 97% and of the coefficient of reproducibility between 79% and 96% in a real population.

Starting as inexperienced coders, our results show a relatively great discrepancy between the two readers, as also between the two readings by

Table II. *Intra-observer error*

Item	Main group code	Sub-group code	No. of sub-groups	Items reported: Once — Total discrepancy	Main group discrepancy	Subgroup discrepancy	Twice	Total no. of items
First 50 ECG tracings coded twice by the same observer								
Nothing reportable	0	—		0	—	—	3	3
Q and QS	1	1-1 to 3-6	21	14	5	9	16	30
Axis items	2	1 to 5	5	3			7	10
High R waves	3	1 to 3	3	4			2	6
ST depression	4	1 to 4	4	11	6	5	12	23
T wave items	5	1 to 4	4	14			20	34
A-V conduction	6	1 to 5	5	2			0	2
Ventricular conduction	7	1 to 6	6				4	5
Arrhythmias	8	1 to 9	9	5			2	7
Miscellaneous	9	0 to 8	8	12			30	42
Last 50 ECG tracings coded twice by the same observer								
Nothing reportable	0	—		0	—	—	4	4
Q and QS	1	1-1 to 3-6	21	5	0	5	26	31
Axis items	2	1 to 5	5	1			6	7
High R waves	3	1 to 3	3	1			5	6
ST depression	4	1 to 4	4	9	4	5	6	15
T wave items	5	1 to 4	4	8			20	28
A-V conduction	6	1 to 5	5	0			0	0
Ventricular conduction	7	1 to 6	6	0			5	5
Arrhythmias	8	1 to 9	9	2			2	4
Miscellaneous	9	0 to 8	8	5			33	38

the same observer. However, there is a tendency to a greater degree of accuracy and reproducibility with increasing experience in use of the code. The improvement in inter-observer error is greatest with regard to ST-T changes, where a discrepancy of 14/19 falls to 6/15 for ST depression and from 19/33 to 9/28 with regard to T changes (Table I). Furthermore, it is important to note that the differences are mostly minor details, i.e. subgroup discrepancies—representing for instance ±0.5 mm in ST or T wave changes (see Tables). Table II shows, for example, that 26 of 31 ECG tracings with codable Q and QS changes are identically coded twice. Only five tracings received different code numbers on the two occasions, the discrepancy being only on the subgroup level with 21 possible subgroups to choose between. A final arbitration easily gave the correct code number. This arbitration was of course performed after the comparative study was completed.

Our study thus confirms previous reports, in that the following criteria should be fulfilled before accepting ECG classification in epidemiological or clinical studies on coronary heart disease.

1. Experience in the use of the Minnesota Code is essential before the coding can be accepted, for example by coding 200 ECGs before the study starts. It is necessary to keep up the training by repeated exercise.

2. There should be at least two observers reading independently and finally reaching the correct code number by discussion or arbitration.

The main ECG findings made by two experienced observers reading the Minnesota Code can thus be accepted as reasonably valid and objective and, as such, used in clinical as well as in epidemiological studies on coronary heart disease.

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Table I. *Age, height weight and diagnoses for glycoside-treated patients*

	[illegible]					
[illegible]	[illegible]		[illegible]		[illegible]	
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]					[illegible]
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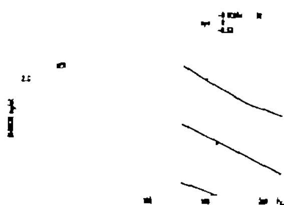


Fig. 2. Regression line with 95% confidence limits for digoxin concentration in plasma on systolic arterial pressure (PSBA) of men at daily dose of 0.125 mg.

clinical status was made and the patients were allocated to one of three groups: insufficient, optimal and toxic glycoside effect. Finally the glycoside concentration was entered. Common statistical methods were applied. Differences between mean values of two groups were tested by *t*-test and differences among several groups were tested by analyses of variance. Differences between variances (variabilities) were tested by *F*-test or Bartlett test. Linear regression analyses were made using glycoside concentration in plasma in ng/ml or in logarithmic units as dependent variable. As independent variables age, height, weight, Hb concentration, ESR, creatinine, the different liver function tests, the relative heart volume, systolic and diastolic BP and time between sampling and last dose were used.

RESULTS

Glycoside concentration values for the dose groups are presented in Fig. 1. Differences were found between the mean values of digoxin in the male dose groups. Those on 0.125 mg daily with mean value of 0.84 ng/ml had a lower concentration than those on 0.25 and 0.375 mg daily for which the mean values were 1.45 and 1.75 ng/ml respectively. For women on 0.125 and 0.25 mg/day the mean plasma concentration levels of 0.96 and 1.88 ng/ml, respectively were not significantly different. For the same dose levels there were no differences between men and women. No correlation between plasma concentration of glycoside and time between last dose and sampling was found in any dose group. At the daily dose of 0.125 mg digoxin to men a negative correlation between systolic BP and plasma concentration was found (Fig. 2). A daily dose of 0.25 mg gave a negative correlation with GOT in men (Fig. 3). At 0.25 mg daily the plasma digoxin concentration for men had a negative correlation with the diastolic BP (Fig. 4). For women no significant correlations were found. Weight had one negative correlation with plasma concentration in dose group 0.375 mg digoxin daily only. Patients with evident glycoside signs in the ECG were compared with those without such changes at the different dose levels, but no differences of plasma concentration were detected. Heart rate with sinus rhythm at rest had no correlation with plasma glycoside concentration at any dose level. Patients treated with diuretics and those not treated showed no difference in plasma glycoside concentrations. No difference was recorded between those with proteinuria and those without. Clinical toxic reaction, AV block I, was found in one man

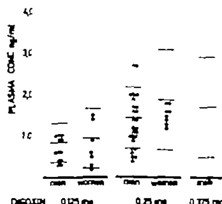


Fig. 1. Distribution of plasma concentration for digoxin in men and women at different dose levels. Mean values and standard deviations indicated by bars.

on 0.25 mg digoxin daily with plasma concentration 1.6 ng/ml. On the same dose two women had toxic reactions with plasma concentrations 0.6 and 3.6 ng/ml, respectively: one had AV block I and a heart rate of 48 beats/min, the second had nausea and visual disturbances. Within the different dose groups analyses of influence from the different diagnoses were made. Those with past myocardial infarction were compared with those without, patients with hypertension with those without, and decompensated patients with compensated. No differences were, however, found for glycoside concentrations. For men on digoxin the variability of plasma concentration at the doses 0.25 and 0.325 mg daily was higher than at the 0.125 mg daily dose. Women had a higher variability than men at a dose level of 0.25 mg.

DISCUSSION

There was a high variability for plasma concentrations at all dose levels. To reduce this variability a fixed interval from the last dose to sampling has been proposed (2, 3, 8, 14). In such investigations the variability has, however, also been great. In the present work the interval from last dose to sampling had no influence on plasma concentration in spite of the wide intervals from 1½ to 51 h. No dose on the last day before the test was taken by 13 patients, which illustrates how difficult it is for a patient to follow medical prescriptions. Some of the patients had the opinion

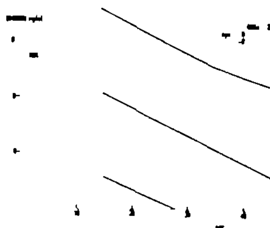


Fig. 3. Regression line with 95% confidence limits for digoxin concentration in plasma on GOT concentration of men at daily dose of 0.25 mg.

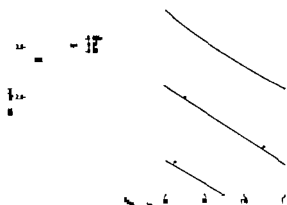


Fig. 4. Regression line with 95% confidence limits for digoxin concentration in plasma on diastolic arterial pressure (PDBA) of men at daily dose of 0.25 mg.

that no medicine should be taken on the day before they were to see the doctor. In another setting we have analyzed geriatric in-patients, where great care was taken to give the dose 6 h before sampling. The variability was, however, of the same magnitude. A daily dose of 0.25 mg digoxin gave a standard deviation of 0.87 ng/ml (15). The influence of age on the sensitivity to glycosides has been discussed (6). In the present material lower doses were prescribed for older patients and they also had lower plasma digoxin concentrations. There were, however, no differences in glycoside reactions in the ECG or according to the clinical evaluation among the older subjects. The present results thus fit with the opinion of higher sensitivity for glycosides at higher ages. In relation to the dose, older patients did not have a high plasma concentration and there was no correlation at any dose level between age and plasma concentration.

The negative correlation of plasma concentration with GOT is hard to explain. If the production of GOT is dependent on mass of muscle or liver and a considerable amount of glycosides is consumed or degraded in these tissues, a lower plasma level for a given dose might be expected. The concentration of digoxin in skeletal muscle is about 10 times higher and in the liver about 20 times higher than in plasma (7). Telling against this mechanism is the lack of correlation between plasma concentration and weight for that dose group. However, weight and mass of skeletal muscle and other glycoside-binding tissues might

have poor correlations in our patients. An influence of weight on plasma concentration was evident only in the highest dose group for digoxin. Of greater interest is the negative correlation with arterial BP in men. Individuals with a higher pressure had lower plasma concentrations. This may be due to a higher consumption of glycosides or to changed relationships between concentrations in plasma and tissues. Those with a higher pressure might have more heart muscle tissue. Heart muscle is known to have about 100 times as high a digoxin concentration as plasma (3). The amount of heart muscle may be better correlated with arterial BP than with heart volume, and this may be the explanation why relative heart volume and plasma digoxin concentration were not correlated.

The present plasma glycoside determinations have not guided us in the choice of dose. The clinical impression of the patients was that they usually had an optimal dose. It has, however been shown that toxic reactions usually indicate high plasma concentrations (2, 10).

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Acta med. scand. Vol. 193, pp. 219–222, 1973

IMMUNOGLOBULINS IN PULMONARY EOSINOPHILOSIS (TROPICAL EOSINOPHILIA)

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Abstract. At the V P Chest Institute of Delhi studies on pulmonary eosinophilosis (tropical eosinophilia) have been going on since many years. The investigations have concerned, among other factors, the pathogenesis of the disease. As regards the pathogenesis and aetiology it is at present being discussed whether infection with human filaria is the original cause of the disease. This microfilaria has, however, not generally been found in the blood or the lung tissue, but it is known that the filarial larvae and microfilariae are rapidly destroyed in the tissues. Immunological studies with the aim of disentangling some obscure points concerning the pathogenesis of the condition are at present performed at the King Gustaf V Research Institute, Stockholm, Sweden. So far it has been found that there exist distinct disturbances of the immunoglobulins. Thus there is a high percentage of IgM, but also an increase of IgG. Studies of isolated fractions of these globulins are in progress.

Pulmonary eosinophilosis (tropical eosinophilia) is a well known clinical entity characterized by cough with scanty sputum and dyspnoea, which may either be paroxysmal or exertional, and massive eosinophilia in the peripheral blood (17). It may be either acute or insidious in its onset, the symptoms persisting for months, even years, if untreated. The condition can be successfully treated either by arsenic injections (20) or by oral administration of diethylcarbamazine (21). The aetiology of the condition has been a matter for speculation ever since its recognition as a separate clinical entity in the thirties. Allergic manifestation (7), mite infestation of the respiratory tract (1, 3, 6, 14), virus infection (9, 10, 19), filarial zoonosis (2, 4, 5), zoonotic ascariasis (11, 12, 13, 16), spirochaetal infection (8), etc. have been put forward as the aetiological basis of pulmonary eosinophilosis.

It is now generally agreed among workers that the disease is due to an infection. That it is not due to bacterial infection has also been agreed by most of the workers. The present author was the first to suggest a virus aetiology. No confirmatory evidence, however, could be found to substantiate such a hypothesis. It is now more or less agreed that the disease is due to a parasitic infection. There are a few significant pieces of evidence to incriminate filaria as the causative factor. Almost all workers who favour a filarial aetiology are of the view that the disease is due to an infection by an animal filaria, possibly Dirofilaria immitis. The main difficulty in establishing the filarial aetiology is the fact that microfilaria is rarely found in the peripheral blood. Those in favour of animal filarial infection have put forward the idea that filarial larvae and microfilariae are rapidly destroyed as a result of a specific hypersensitivity reaction after they are caught in the pulmonary capillaries. Hence no organisms are found in the peripheral blood.

If therefore, the theory of filarial aetiology is to be substantiated, other evidence, preferably of an immunological nature, must be provided. It was with that end in view that the present study was undertaken.

PRESENT STUDY

In order to determine immunoglobulin changes in the sera of patients with pulmonary eosinophilosis, samples of sera from 8 patients were examined by ultracentrifugation. Similar studies were made on samples of sera from patients suffering from either elephantiasis or from other manifestations of filariasis. The ultracentrifugations were performed at the King Gustaf V Research Institute, Stockholm, Sweden, under the direction of Professor N. Svartz.

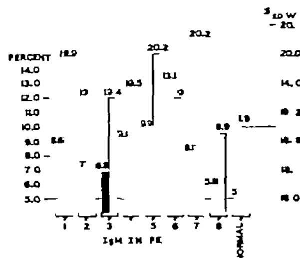
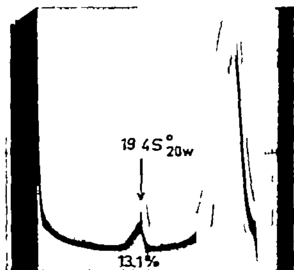


Fig. 1. Values of the sedimentation constant (S_{20w}) for IgM in 8 cases of pulmonary eosinophilosis. The relative percentage of IgM is 5.8 in one case but above that in all other cases.



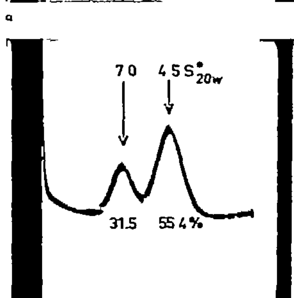


Fig. 3. Ultracentrifugation of serum from case of pulmonary eosinophilosis (a) after 30 and (b) 90 min. Note that the IgM percentage is as high as 13.1 and the IgG percentage 31.5. The sedimentation constant of IgM is 19.4.

RESULTS

Figs. 1, 2 and 3 indicate values for IgM and IgG in cases of pulmonary eosinophilosis.

It is interesting to note that IgM shows a significantly increased percentage in all 8 sera tested and that the sedimentation constant, except in one case, is above 19. Controls of 5 new sera gave the same result. It has been pointed out by ...z (15) that, when IgM is distinctly increased in any particular disease, a pathological type of macroglobulin is usually involved. Our studies have clearly shown that the macroglobulin in pulmonary eosinophilia does not belong to the haem ggl tinating variety like the rheumatoid factor. It is also particularly interesting that neither the whole sera containing IgM, IgG and albumin nor the isolated fractions react with common IgE. A number of commercially available anti-IgE were tested against the sera and

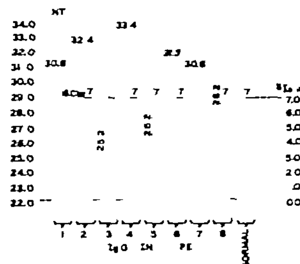


Fig. 2. IgG percentages and sedimentation constants in the same 8 cases of pulmonary eosinophilosis. Note that the IgG percentage is above 25.2 in all cases.

their immune fractions. All of them were found to be negative. A new series of experiments will be performed by the method of Wide et al., recently made available from Pharmacia International, Uppsala, Sweden.

It is of interest to note from Fig. 2 that the percentages of IgG are also markedly raised.

It is significant that the values for IgM and IgG in the 8 sera from cases of filariasis were higher than normal (Figs. 4 and 5), and the sedimentation constant was also abnormal, i.e. above 19. It is therefore reasonable to assume that the abnormal macroglobulin found in pulmonary eosinophilosis is probably the same as that found in human filariasis.

DISCUSSION

The macroglobulin levels are significantly raised in pulmonary eosinophilosis and in filariasis. Sedimentation constants are more or less of the same level in both groups. One is tempted to assume that the rise in IgM in sera of pulmonary eosinophilosis and of filariasis is due to the same, or antigenically similar aetiological agents.

In another study (18) it has been found that the leucocyte adhesion phenomenon, by which leucocytes destroy microfilariae is observed more frequently with M. bancrofti than with D. immitis or D. repens. Since human filariasis is due to W. bancrofti infection, pulmonary eosinophilosis, in which the same form of immunological reactions develop, may also be due to this same aetiological agent.

The question arises as to why human filarial infection produces the manifestation of pulmonary eosinophilosis in some people while in others it produces the typical manifestation of filariasis. It may be that in patients suffering from pulmonary eosinophilosis a specific immunological status is acquired, as a result of which microfilariae reaching narrow pulmonary vessels are unable to pass through because of leucocytes and platelets adhering to them. Immunological changes which occur on the walls of small pulmonary blood vessels cause the development of miliary granuloma, eosinophilia and respiratory symptoms, which are characteristic of pulmonary eosinophilosis.

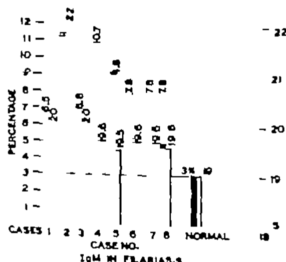


Fig. 4. IgM values in 8 cases of filariasis. Note that the sedimentation constant is above 19 in all cases. IgM percentage is 6.5 and above.

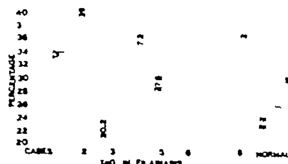


Fig. 5. IgG in the same 8 cases of filariasis. IgG percentages are above 22 in all cases, except one.

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Acta med. scand. Vol. 193 pp. 223–226, 1973

ACQUIRED HAEMOLYTIC ANAEMIA

I. *Incidence and Aetiology*

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Abstract. Acquired haemolytic anaemia has been studied during a 5-year period in one of Sweden's health care regions. Altogether 161 patients were found, corresponding to an overall incidence of 3 (2.6) patients per 100 000 and year. Women predominate (63%), especially in the age groups 15–34 years, in which 37% of the female patients with acquired haemolytic anaemia were or had recently been pregnant. Mechanical factors are discussed as possible causes of haemolysis occurring during or immediately after pregnancy.

Acquired haemolytic anaemia is a condition with an unusually varied aetiology. Many causes have been known for a long time, others have only recently been identified (5), still others remain obscure or incompletely established (3). As a background for a study of drug-induced haemolytic anaemia, we have analysed the incidence and the aetiology of acquired haemolytic anaemia, occurring during a 5-year period in one of Sweden's health care regions. The purpose is not to discuss the detailed mechanisms of haemolysis, but to analyse the incidence and the main causes as they appear from the hospital records.

MATERIAL AND METHOD

Since 1964 discharge diagnoses from all hospitals within the Uppsala health care region (1.2 mill. inhabitants = 15% of the Swedish population) have been computer recorded, making it possible in each case from the data lists to find the hospital, the department, the time of hospitalization, the individual and the diagnoses. We have studied the lists for the 5-year period 1964–68 and, through the courtesy of the head physicians of the various departments, have been permitted to scrutinize the medical records for the patients. Unfortunately it has not been possible to retrieve all the records. The year of the first hospitalization, the age and sex of the patients have, however, been obtained from the data lists. The missing records (n = 18, 11%) have been analysed with regard to year of occurrence as well as to age and sex of the patients and been found to have the same distribution as the available records. Thus, there are no indications that any systematic error would arise from the omission of these records from the discussion of aetiology.

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RESULTS

In the Uppsala region there occur approximately 30–35 (mean 32) cases of acquired haemolytic anaemia each year (Table I)—with only small yearly variations. The observed frequency would correspond to 200–230 cases in Sweden each year. During the same period and in the same region 59 patients were found with hereditary haemolytic anaemia (12 each year), corresponding to a yearly frequency of 80 patients in Sweden. Thus, in Sweden at the present time, acquired haemolytic anaemia seems to be 2–3 times as common as the hereditary forms.

The distribution with regard to sex and age, as well as the incidence, are given in Table II and Fig. 1. Women predominate in all age groups with the exception of the youngest (0–14 years), in which the sex distribution is even. In the age groups 15–34 years there is a more marked predominance of women than at other ages, probably due to the high frequency of pregnancies in these age groups (37%). In both sexes a marked increase is found in the number of cases after the age of 50.

The causes of acquired haemolytic anaemia have been listed in Table III, which comprises 89% of the total number of patients. The largest group is the "idiopathic", i.e. haemolytic anaemia of unknown origin. Autoimmune mechanisms are

haemolysis as an additional cause of anaemia during pregnancy

The role of viral infections in producing haemolytic anaemias has not been clarified. It seems as if the virus could alter erythrocyte surface structure enough to produce an autoimmunization. Three patients were thought to have haemolytic anaemia caused by virus infection, a 4-year old girl died of a varicella infection, accompanied by haemolytic anaemia—an immunopathic condition, however had previously been suspected in this patient. A 1 year-old boy died with a rapid course of haemolytic anaemia—postmortem examination of the brain indicated the existence of viral encephalitis. Finally a 66-year-old woman suffered from an acute infection, probably of viral origin with haemolytic anaemia, splenomegaly and erythema multiforme—when examined two years later she was completely healthy

Among the malignant conditions accompanied by haemolytic anaemia, the malignant lymphomas —as always—predominate. From many aspects these cases should be included under the general heading autoimmune haemolytic anaemia. Haemolytic anaemia has been reported to occur in conjunction with ovarian tumours (9) most of which were dermoids or teratomas—again a finding that makes one wonder whether mechanical factors may not be of pathogenetic importance.

The drugs most commonly involved as the cause of haemolytic anaemia, in this material, were sulfonamides and methyldopa. Methyldopa is especially interesting as it causes an autoimmune state with antibodies not against the drug but against erythrocytes. In a group of drug-induced haemolytic anaemias, reported to the Swedish Adverse Drug Reaction Committee during 1966–70 (1) methyldopa was by far the most common cause and was responsible for 47% of all reported cases of drug-induced haemolytic anaemia. The number of methyldopa-induced cases in that study seemed to be increasing.

Altogether 6 deaths have been registered in connection with hospitalization for acute haemolytic anaemia in this material. In 2 of these 6 cases the cause of death most likely was a virus infection (see above) and not the haemolysis per se. In four instances (patients aged 58, 67 78 and 86 years, respectively) the cause of death seems to have been an acute haemolytic attack that could not be stopped by steroids or balanced by transfusions.

A large group of patients (48 = 34%) were listed as having haemolytic anaemia of unknown origin ("idiopathic") Although the patients in this group were somewhat older no significant differences were found with regard to age and sex in this group as compared to the rest of the material.

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Acta med. scand. Vol. 193 pp. 227–231 1973

ACQUIRED HAEMOLYTIC ANAEMIA

II. *Drug-induced Haemolytic Anaemia*

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Abstract. Forty-five cases of drug-induced haemolytic anaemia have been reported to the Swedish Adverse Drug Reaction Committee during the 5-year period 1966–70. Methyldopa was responsible in 21 cases (47%) and caused the only two deaths. Sulfonamide preparations for long-term use induced haemolysis in 10 patients.

Drug-induced haemolytic anaemia is a comparatively rare condition. Two basic mechanisms are involved, one a direct toxicity to sensitive erythrocytes (toxic haemolysis), the other an immunological mechanism. Toxic haemolysis occurs without sensitization and generally is due to a defect in the erythrocytes, as e.g. in the most frequent type, glucose-6-phosphate dehydrogenase deficiency. The most common variety of the immune type of drug-induced haemolytic anaemia in recent years seems to be the one induced by methyldopa, where a clinical picture of autoimmune type is produced in which, in several instances, the haemolytic anaemia is combined with fever, hepatocellular damage (10) and other autoimmune manifestations such as LE cells (9) and antinuclear factor (4).

The present paper is an analysis of the cases of drug-induced haemolytic anaemia reported to the Swedish Adverse Drug Reaction Committee during a 5-year period.

MATERIAL

All patients reported to the Swedish Adverse Drug Reaction Committee during the period Oct. 1965 (when the Committee was established)–Dec. 31 1970, have been included. Altogether 9 patients who were erroneously listed as having haemolytic anaemia have been excluded. These patients had agranulocytosis (1 pat.), sideroachrestic anaemia (1 pat.), cutaneous allergy (1 pat.), uncertain connection with cytostatic drug treatment (1 pat.) and toxic hepatitis (5 pats.). The remaining material consists of 45 patients.

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RESULTS

The number of cases with drug-induced haemolytic anaemia varies from year to year, but it seems as if it would be increasing. The total average at the beginning of the 5-year period was 7 patients per annum, at the end 12 patients per annum (Table I).

The age and sex distribution is given in Table II—the age-related incidence in Fig. 1. There is found to be a marked predominance for women, who constitute 84% of the total material. The incidence rises with age.

The drugs that cause haemolytic anaemia are shown in Table III. Methyldopa is responsible for almost half of the cases (21/45 = 47%) with two deaths. Sulfonamide preparations follow with 10 patients, 5 from salicylazosulfapyridine and 5 from a sulfonamide combination used for chronic urinary tract infections: Sulfapral® = (sulfamethizole + sulfamethoxipyridazine). Three patients contracted haemolytic anaemia from a sulphone preparation (diaphenylsulphone). The remaining cases were caused by a variety of drugs, one case only from each drug.

The blood values in most patients have been normalized after discontinuation of the offending drug. Only in 12 patients (27%) had more rigorous measures to be taken. Ten patients were given corticosteroids, with prompt effect in nine. The last patient, whose anaemia was caused by a combined preparation (Bellergal® retardatum, Sandoz) did not respond to steroid treatment and

Table I. *The yearly incidence of drug-induced haemolytic anaemia reported to the Swedish Adverse Drug Reaction Committee*

	Methyl-dopa	Other drugs	Total
1966	1	1	2
1967	5	7	12
1968		4	6
1969	5	6	11
1970	8	6	14
	21	4	45
1971	6		

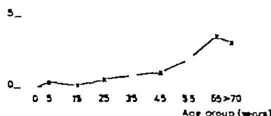


Fig. 1. Age-related incidence of drug-induced haemolytic anaemia (men and women).

was splenectomized with good result. Two patients had such massive haemolysis that anuria developed. Both were treated by haemodialysis. One of the patients died from ventricular tachycardia after 4 treatments although the haemolysis seemed to diminish (case 2—see below), the other made a complete recovery.

The doses given and the medication time for the methyldopa cases are given in Table IV.

Altogether two deaths occurred, both from methyldopa-induced haemolysis (see Discussion).

DISCUSSION

All individuals may have haemolysis when exposed to sufficiently high concentrations of a potentially haemolytic drug. Nevertheless drug-induced haemolytic anaemia is a rare condition—a contradiction that so far has not been satisfactorily explained. The mechanisms underlying the various forms of drug-induced haemolytic anaemia have been reviewed by Dausset and Contu (7), by Beutler (1) and, for the immune cases, by Worlledge (13). There are two types of drug-induced haemolytic anaemia on an immunological basis: the "immune" type caused by a number of drugs and the "autoimmune" type provoked by methyldopa and, in one report (11) by the antirheumatic drug, mefenamic acid. The mechanism behind these types of haemolytic anaemia will not be discussed here.

Table II. *Drug-induced cases of haemolytic anaemia, by sex and age*

Age group	Men	Women	Total in 5 y
0–4	—	—	—
5–14	—	2	2
15–24	—	1	1
25–34	—	3	3
35–44	1	3	4
45–54	2	4	6
55–64	3	7	10
65–69	—	7	7
70	1	10	11
	7	37	44
Age unknown	—	1	1
	7	38	45
	16%	84	100%

Methyldopa dominates the present material and was responsible for almost half (47%) of the cases reported to the Swedish Adverse Drug Reaction Committee. It seems as if the number of cases would be increasing, although it is difficult to draw conclusions from figures as small as ours. Besides we know that adverse reactions are grossly underreported (12). Methyldopa-induced haemolytic anaemia now seems to be regarded as such a well known and established drug complication that according to Swedish regulations it is no longer necessary to report the cases. If the number of reports nevertheless is constant or increasing, that probably means that the frequency really is increasing.

The clinical picture in the methyldopa patients is rather monotonous—the patients gradually develop a moderate anaemia (mean low value 8.6 g/100 ml), with low or absent haptoglobin and with reticulocytosis, and often show slightly to moderately increased serum bilirubin values. The direct, and generally also the indirect, Coomb's test is positive. In a few instances fever develops and/or signs of toxic hepatitis.

The medication time varies from a few months to several years (Table IV). Only two patients, however had a shorter exposure time than 6 months. One man, 45 years old, had been given an unusually large dose (0.50 g × 3). The other a 61-year-old woman, had a long-standing pre-existing but clinically fully compensated hereditary spherocytosis. She was given methyldopa in a moderate dose (0.25 × 3) and already after 3 months was hospitalized in a bad condition with fever increased haemolysis and jaundice. A provocation test later confirmed that methyldopa was responsible for that acute condition.

Two deaths have occurred in connection with methyldopa-induced haemolysis.

Case 1

A 50-year-old woman was hospitalized in comatous and hemiparetic condition. She had suffered from hypertension for at least 10 years and for the last 3 years had been treated with methyldopa 0.25 g 2 and chlorthalidone 0.05 g 1 Her Hb on admission was 8.6 g/100 ml day later 7.0 g/100 ml, and ESR 143 mm/h. Her direct Coomb test was strongly positive. She was treated as case of drug-induced haemolytic anaemia with ACTH but died after 3 days.

Although she suffered from cerebral vascular accident, it was felt that her rapidly developing haemolytic anaemia contributed to her death.

Table III. *Drugs causing haemolytic anaemia*

	Men	Women	Total
Methyldopa	3	18	21 (2 deaths = 10 %)
Sulfonamides			
Salicylazosulfa-pyridine	—	5	
(Salazopyrin Azulfidine®)			
Sulfamethizole + sulfamethoxypyridazine (Sulfapral®)	1	4[b]	10
Diaphenylsulphone	—	3	3
Indomethacin	—	1	1
Chlorthalidone	—	1	1
Nitrofurantoin	—	1	1
Phenylbutazone	—	1	1
Apresolin	1	—	1
Benzyl penicillin	—	1	1
Verapamil	—	1	1
Clopenthixol	—	1	1
Several drugs[d]	2	1	3
	7	38	45

Overdose (1 g 4), unintentionally given.

T patients had renal insufficiency with serum creatinine alues 3.8 and 11.5 mg/100 ml, respectively

High dosage—40 mill. units daily (septicaemia) during 14 days.

1) Bellergal retardatum (Sandoz)—total belladonna alkaloids + ergotamine + phenemal. 2) Phenacetin acetylsalicylic acid in two combined preparations. 3) Heavy antiepileptic medication with diphenylhydantoin + primidone + phenemal.

Table IV *Dose and medication time in methyldopa haemolytic anaemia*

	No. of pats. (n)	( )
Total daily dose (g)		
0.25	1	5
0.50	6	29
0.75	10	48
1.00	2	10
1.25	—	—
1.50	2	10
	21	102
Medication time (mo.)		
0– 6	2	9
7–12	6	29
13–18	4	19
19–24	5	24
24	4	19
	21	100

Dose and medication time for two patients with exposure time 6 months.
1.50 g – 1 mo.
0.50 g – 2 mo. (pre-existing compensated hereditary spherocytosis).

Case 2

A 58-year-old woman, previously healthy had since July 1965 been treated for an incidentally discovered moderate arterial hypertension with methyldopa 0.25 g 2 and chlorthalidone 0.05 g 1. She suddenly fell ill on April 16, 1966, with low back pain and, on the following day she passed dark urine and ran high fever. She was admitted to hospital on April 17 Her Hb was 7.7 g 100 ml, WCB 21 000 and platelets 130 000 Serum bilirubin was 3.4 mg/100 ml and serum creatinine 8.5 mg 100 ml, going up the following day to 15 mg/100 ml. Haemodialysis was started but was repeatedly complicated by fall in arterial BP and by cardiac arrhythmias of various types. Free plasma Hb and creatinine, however, decreased, but during the fourth dialysis treatment she had cardiac standstill and died. Postmortem examination showed an allergic haemolytic reaction especially in the kidney with fibrinoid necrosis in the vessels in the glomeruli and haemorrhages as well as erythrocyte and Hb casts in the tubular lumina. Histological signs of angiitis ere found also in the small vessels in the cerebrum and the intestines.

The case was interpreted as an allergic reaction to methyldopa. Coomb direct test was positiv

Both the patients had been treated for long periods with moderate doses of methyldopa before the rapidly fatal haemolysis became clinically apparent. No special circumstances could be found that brought about the acute disease, although it has been suggested that additional factors would be necessary for the development of methyldopa haemolysis.

All brands of methyldopa at present used in Sweden are represented in the material, the l-form as well as the racemic variety.

A positive direct Coombs's test in patients treated with methyldopa was first reported in 1966 (5). The number of reported cases with positive immunological reactions and/or overt haemolytic anaemia has risen rapidly and the total number of methyldopa-induced autoimmune haemolytic anaemia cases already in 1969 exceeded that of the total of all drug-induced "immune" haemolytic anaemias (14). Worlledge et al. (14) estimated the risk of autoimmune haemolytic anaemia on methyldopa treatment at 0.02%. We have made an estimate from the known sale of methyldopa in one part of Sweden and from the reported cases of methyldopa-induced haemolytic anaemia and arrived at exactly the same figure, 1:6000 or 0.02% (3). As, on the average, only one third of occurring drug complications are reported to the Adverse Drug Reaction Committee in Sweden—and haemolytic anaemia from methyldopa probably even more rarely—the figure must be regarded as an absolute minimum.

Regarding treatment, it has been suggested that corticosteroids should be given only if cessation of methyldopa treatment alone is not sufficient and if the haematological condition of the patient deteriorates (8). In view of the benign course in most patients this advice seems well founded, although the fear of early administration of corticosteroids seems slightly exaggerated.

Apart from methyldopa, sulfonamides are the only group of drugs which cause a significant number of haemolytic anaemias. It is noteworthy that only two rather specific sulfonamide preparations are involved, both being used for long-term treatment. Haemolytic anaemia with Heinz body formation during treatment with salicylazosulfapyridine was reported as early as 1958. Although the frequency does not seem to be high (2) several cases have been reported later. Sulfapral® is a combination of a short-acting (sulfamethizole) and a long-acting (sulfamethoxipyridazine) sulfonamide intended for long-term use in chronic urinary tract infection. The preparation has been widely used in Sweden during the last decade. It should be noted that an overdose was given in 3 of the 5 Sulfapral patients. One man was—by mistake—given twice the regular dose. Two patients had renal insufficiency with elevated serum creatinine values (Table III) but were nevertheless treated with standard doses. With the exception of the sulphone preparation diaphenylsulphone (Dapsone) used for treatment of dermatitis herpetiformis, in this study no other drug caused more than a single reported case of haemolytic anaemia.

With a knowledge of the number of all cases of acquired haemolytic anaemia occurring during the years 1966–68 it is possible to find the proportion that has been reported to the Swedish Adverse Drug Reaction Committee. The figure turns out to be 25% and is thus of the same order of magnitude as in drug-induced thrombocytopenia (33%), agranulocytosis (31%) and aplastic anaemia (34%) (3).

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Acta med. scand. Vol. 193 pp. 233–245 1973

MYOCARDIAL METABOLISM OF EXOGENOUS PLASMA TRIGLYCERIDES IN RESTING MAN

Studies during Alimentary Lipoemia and Intravenous Infusion of a Fat Emulsion

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Abstract. A nephelometric technique has been used to measure concentrations of exogenous triglyceride in arterial and coronary sinus blood. The technique has been validated in comparison of estimated arterial-coronary sinus differences with those obtained by the chemical determination of total plasma triglyceride. Statistically significant disappearances of exogenous triglyceride across the coronary circulation were observed both during the lipaemia after fatty meal and during the i.v. infusion of fat emulsion and glucose solution. The uptake of exogenous triglycerides, about 6% of the arterial concentration, could supply about 50% of the heart's energy requirements. Significant productions of glycerol across the coronary circulation were found in both types of fed state in amounts corresponding to complete lipolysis of 25–50% of the exogenous triglyceride removed. Net myocardial extractions of free fatty acids were less than in previous study in the fasting state, which may mean that all triglyceride fatty acid released by lipolysis is not taken up by the myocardium. Also, myocardial extraction of blood glucose was less in the oral fed state than in previous study in the fasting state.

Dietary fatty acids containing more than 12 carbon atoms are, after absorption, delivered into the circulation from the thoracic duct as large triglyceride-rich fat particles called chylomicra (41). The metabolism of chylomicra has been studied in great detail in experimental animals. Direct uptake of chylomicra or of chylomicron triglycerides (exogenous triglycerides) by liver (32) and adipose tissue (37) as well as lipolysis (14, 18) are the mechanisms believed to result in clearance of chylomicra from blood. Peripheral tissues could obtain chylomicron fatty acid following lipolysis remote from the tissue (18), especially when lipoprotein lipase activity appears in the blood following i.v. heparin (15, 33), or as a result of local lipolysis at tissue vessel walls (35, 40) or cells (19).

Soon after the injection of labelled chylomicra into rats radioactivity can be demonstrated in the myocardium (7). Furthermore, the isolated heart, from the rat or the rabbit, assimilates and oxidizes chylomicron fatty acids when perfused with labelled chylomicra (12, 13, 27, 38, 42). The rat heart contains lipoprotein lipase (26). This is also the case for the human heart (33, 39). In man, however, uptake of exogenous triglycerides by the heart has not been demonstrated. Yet a very small arterial-coronary sinus (a-cs) difference in exogenous triglycerides could contribute significantly to the metabolic requirements of the human heart. Extraction of endogenous plasma triglycerides by the human heart has recently been demonstrated using precise measurements for the small a-cs difference in triglyceride concentration (8). The aim of the present study was to use precise nephelometric and chemical methods to discover whether exogenous plasma triglycerides are taken up by the human heart.

MATERIAL AND METHODS

Subjects

Ten male volunteers between the ages of 22 and 38 were studied. No subject gave past history of cardiovascular or metabolic disease or was on continuous medication. All were free from symptoms referable to the cardiovascular system or suggestive of metabolic disorder, and all had normal resting as well as exercise ECG. However, two subjects (nos. 3 and 10) were found to have mild type II A hyperlipoproteinaemia according to the WHO classification (1).

Design of the studies

All subjects were investigated without sedation after an overnight fast. That they had fasted was verified by the absence of chylomicra on paper electrophoresis and by the finding of a blood sugar concentration below 1 000 mg/l. They had received an oral dose of iodine in the form of Lugol's solution followed by an i.v. injection of 5–8 µC ^{131}I-albumin (provided by G. Birke and L. O. Plantin, King Gustaf V Research Institute, Stockholm, Sweden) 2 days prior to the study in order to detect change of plasma water concentration across the coronary vascular bed. A short teflon catheter was inserted into the right brachial artery for blood sampling. A specially designed radio-opaque teflon catheter was then introduced percutaneously into the coronary sinus, also for blood sampling, from a left arm vein. In the first two series of studies (see below), from a vein in the right arm, the tip of another teflon catheter was advanced to the superior vena cava for the constant infusion of glucose and a short catheter was inserted into another antecubital vein for infusion of the emulsion Intralipid®-S. The subjects rested supine throughout the experiment. Heparin was not administered. Instead, the arterial catheter was kept patent by intermittent flushing with isotonic saline and the coronary sinus catheter by continuous infusion of 0.5% citrate in isotonic saline at a rate of about 50 ml hourly.

The studies were of three types (Fig. 1). In the first two a modified form of Intralipid® identified as Intralipid®-S, was infused to study the myocardial extraction of exogenous plasma triglycerides in an artificially induced steady fed state. Intralipid® (10%), used extensively for i.v. nutrition, is an emulsion consisting of soybean oil (10 g%) and egg yolk phospholipid (1.2 g%) stabilized and made isotonic with glycerol (2.5 g%). The glycerol of the emulsion Intralipid® would have changed the normal plasma glycerol concentration pattern during clearance of exogenous triglycerides and, therefore, Vitrum, Stockholm, Sweden, supplied a 10% fat emulsion with 5 g% sorbitol instead of glycerol, prepared by Dr I. Håkansson. Chemical analysis of the emulsion showed the free fatty acid (FFA) concentration to be 1 700 µmol/l and the glycerol concentration 800 µmol/l. Since the total amount of emulsion administered during an experiment was less than 100 ml/hour corresponding to about 1–2% of the endogenous turnover of these substrates, the amounts of FFA and glycerol were not expected to alter plasma concentrations significantly.

In the third series of studies chylomicron uptake was determined after a meal rich in fat, i.e. a "naturally induced fed state".

In the *first series* (4 subjects) the myocardial extraction of triglyceride was measured both by a nephelometric and a chemical method in order to compare the techniques. After arterial blood had been taken for the nephelometric plasma blank (5×), for fasting blood glucose (2×), for fasting plasma triglyceride (2×) and plasma cholesterol (2×) determinations and for paper electrophoresis of lipoproteins, a single injection of 10% Intralipid®-S, 0.1 g/kg b.wt. was given. (A× indicates that A replicates were taken from pooled plasma, with the exception of glucose for which A replicates were taken from whole blood.) This was followed by constant i.v. infusions of 20% glucose and 10% Intralipid®-S at known rates of 1.55–1.66 ml/min. Blood was sampled simultaneously from the artery and the coronary sinus after 1, 2, 3 and 4 hours of simultaneous infusion of glucose solution and triglyceride emulsion and analysed nephelometrically for triglyceride-rich particles (10×), chemically for triglyceride (10×3×) and chemically for glycerol (4×4×). (A×B× indicates that A replicate plasma or whole blood samples were taken and that each sample was analysed B times.) In one subject (no. 3) arterial and coronary sinus plasma obtained at 1, 2, 3 and 4 hours was examined electron microscopically.

In the *second series* (3 subjects) an "intravenous fed state" was achieved as described for the first series (Fig. 1). Before the infusions were begun, blood was taken for the nephelometric plasma blank (5×), for determination of glucose (2×), FFA (2×), glycerol (3×4×), triglyceride (2×) and cholesterol (2×) and for paper electrophoresis of lipoproteins. After 1, 2, 3 and 4 hours of infusion, blood samples were taken for nephelometry (10× of arterial and of coronary sinus blood at all intervals) and for the determinations of glucose (2× of arterial blood after 1 and 3 hours, 10× of arterial and of coronary sinus blood after 2 and 4 hours) and FFA (2× of arterial and of coronary sinus blood after 1 and 3 hours, 4× of arterial and of coronary sinus blood after 2 and 4 hours). After 2 and 4 hours only arterial and coronary sinus blood was taken for glycerol (4×4×), ^{131}I-albumin (10×) and oxygen saturation and content determinations.

In the *third series* (3 subjects) an oral fed state was achieved by giving the fasting subjects a standardized meal at the onset of the experiment (Fig. 1). Fat was given as 200 ml cold cream (40% fat) and 15 g cheese (15% fat). Carbohydrate was given in the form of 15 g cheese (15% carbohydrate) and 50 g bread (50% carbohydrate). The calorie ratio carbohydrate : fat was therefore about 1 : 6. Before the meal arterial blood was taken for the nephelometric plasma blank (5×), for determination of glucose (2×), FFA (2×), glycerol (3×4×), triglyceride (2×) and cholesterol (2×) and for paper lipoprotein electrophoresis. At 3 and 4 hours after the meal, when maximal alimentary lipaemia was expected, light scattering intensity (LSI) by nephelometry (10×), glucose (10×2×), FFA (4×) and glycerol (4×4×) concentrations, ^{131}I-albumin (10×) radioactivity and oxygen saturation and content were determined on samples of arterial and coronary sinus blood drawn simultaneously; total triglycerides (2×) were determined on arterial samples only.

Treatment of samples

Paired samples of arterial and coronary sinus blood were drawn into heparinized glass syringes for the estimation of oxygen saturation and content. The other blood samples were drawn into unheparinized plastic syringes, then immediately transferred to plastic tubes containing lyophilized heparin and placed in an iced water bath. Aliquots for the estimation of blood glucose were taken from paired arterial and coronary sinus samples and deproteinized with perchloric acid within

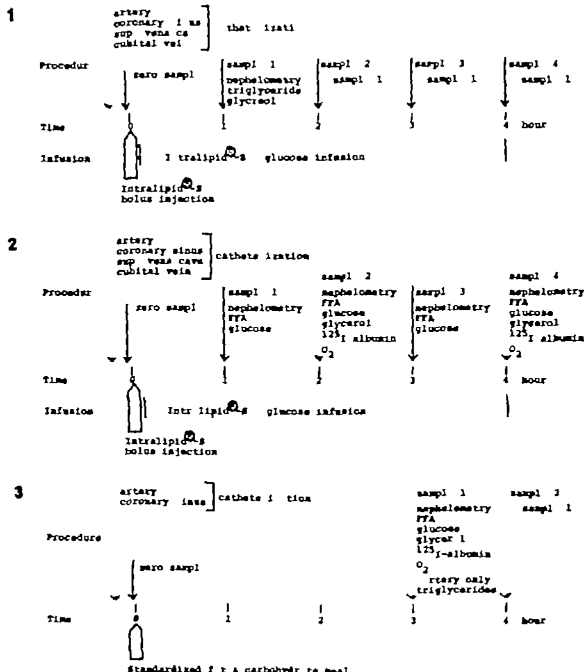


Fig. 1 Design of the studies. Zero time as taken as either the commencement of the infusions or the beginning of the meal.

5 mls of the blood being drawn. The heparinized arterial or coronary sinus blood was then pooled and thoroughly mixed. One portion was centrifuged at +4°C for 15 min at 1 500 g and the plasma separated. Plasma samples ere kept in iced water and extracted ithin 4 hours of being draw with isopropanol for chemical determination of arterial plasma triglyceride concentration in the fasting state or in the oral fed state at 3 and 4 hours after the meal. Extractions with the Dole mixture for chemical determination of plasma FFA were made ithin 2 hours on plasma kept at +4°C until that time. Aliquots of the arterial and of the coronary sinus plasma

Table I. *Comparison of nephelometric and chemical methods for estimating differences in triglyceride concentration across the coronary circulation and the corresponding a-cs differences in glycerol concentration during infusion of Intralipid® S (means ± S.E.M.)*

The percentage of myocardial extraction in relation to arterial concentration. In the case of the chemical method the arterial triglyceride concentration prior to the fat emulsion infusion has been subtracted from the arterial total triglyceride concentration to give the arterial exogenous triglyceride concentration. (This assumes that the arterial endogenous triglyceride concentration has not changed during the infusion)

Subject no.	Sample no.	a-cs difference: Triglyceride: Chemical (μmol/l)	(%)	Nephelometric (μmol/l)	(%)	Glycerol (μmol/l)
1	1	19±59 ns	0.6	−90±20*	−3.4	−7±1
	2	−86±40	−2.1	−30±20 ns	−1.0	−3±1
2	1	138±30	6.2	90±20	4.5	−32±3
3	1	55±49 ns	2.5	70±10	4.1	−24±3
	2	34±33 ns	1.5	70±20	3.8	−37±2
	3	73±36 ns	2.8	50±20	2.6	−41±1
	4	93±28	3.5	50±20	2.5	−47±2
4	1	104±14	3.6	160±20	7.5	−29±8
Mean ±S.E.M.		54±24	2.3±0.9	46±27	2.6±1.2	−[illegible]8±6

ns, $p > 0.05$ $p < 0.05$ $p < 0.01$ $p < 0.001$ (based on *t*-test of difference between t[illegible]o means).

and stored at −20°C for determination, usually within one week, of plasma free glycerol. The other portion of heparinized arterial or coronary sinus blood was centrifuged at room temperature for 10 min at 60 *g* (9). Plasma was pipetted off, recentrifuged under the same [illegible]ions and separated again. From this plasma aliquots [illegible] taken for nephelometry. I[illegible] the first series, centri[illegible]ion at 60 *g* was carried out at +4°C and the separated plasma kept in iced water. Here the plasma was [illegible]lso extracted with isopropanol within 4 hours of being dra[illegible] for chemical triglyceride determination and frozen [illegible] [illegible]20°C for subsequent determination of glycerol.

Chemical determinations

Plasma triglyceride concentration was determined by measuring total plasma glyceride-glycerol, by an Auto Analyzer technique (25). The [illegible]alues [illegible]ere corrected for free glycerol, which is measured together with glyceride glycerol by this method (3[illegible] 5). Plasma free glycerol was measured by an enzymatic fluorimetric method (11). There [illegible]as no detectable difference in plasma glyceride-glycerol concentration between plasma extracted within 1 ho[illegible] and at 4 hours after blood was dra[illegible] [illegible]hen plasma was kept in iced water until extraction. Total plasma cholesterol was also determined by an Auto Analyzer technique (46). Plasma FFA concentrations were measured by the method of Trout et al. (48). Serum lipoproteins were separated by paper electrophoresis according to Lees and Hatch (30). Blood glucose was measured by [illegible] glucose oxidase method according to Hjelm (20). The oxygen saturation was measured spectrophotometrically (22). The oxygen content was calculated from the oxygen saturation, the Hb concentration and the oxygen tension measured with [illegible] polarographic electrode (Instrumentation Lab. Mod. 113).

Radioisotope determinations

[131]I-albumin was given as a tracer for plasma albumin in order to determine whether any systematic differences existed between arterial and coronary sinus blood [illegible]hich might indicate haemoconcentration or haemodilution, since this [illegible]ould affect estimates of myocardial substrate extraction based on a-cs concentration differences. [131]I radioacti[illegible]ity [illegible]as determined on replicates of arterial and of coronary sinus plasma (3).

Nephelometric determinations

The concentration of the exogenous triglycerides in plasma after the fat-rich meal or after the infusion of the artificial triglyceride emulsion Intralipid®-S was determined using a nephelometric technique (9). By exogenous lipids we mean either lipids as chylomicrons entering blood from the thoracic duct during absorption of dietary fat or lipids as artificial fat emulsion. If these lipids reappear in plasma after once having left the blood stream they are called endogenous lipids. Endogenous plasma triglycerides may also be synthesized from carbohydrates and from FFA. An Intralipid® S standard curve was dra[illegible]n as described for Intralipid® (9). When the LSI read[illegible]ng the Thorp micronephelometer Type 236 (Scientific Fur[illegible]nishings Ltd., Poynton, Cheshire, England) [illegible]as plotted against the concentration of the Intralipid®-S fat, a linear standard cur[illegible]e was obtained. From each arterial and coronary sinus plasma sample [illegible]5 or 50 μl, depending on the degree of lipaemia, was pipetted into 5 ml saline con-

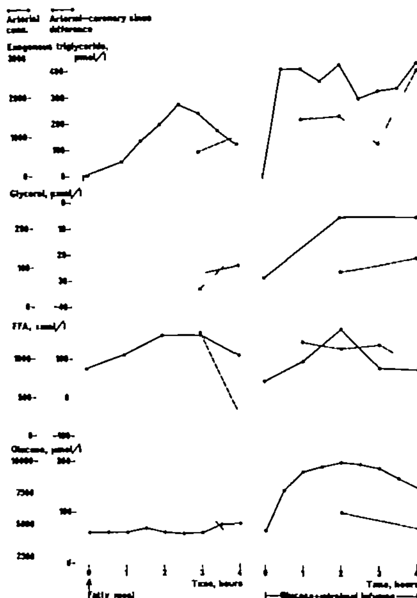


Fig. 2. Arterial concentrations (— left scale) and a-cs differences (- - right scale) in exogenous triglycerides (µmol/l plasma), glycerol (µmol/l plasma), FFA (µmol/l plasma) and glucose (µmol/l blood), in one study during alimentary lipaemia (left) and one during the i.v. infusion of Intralipid® and glucose (right).

times. The 20 samples from an a-cs pair were randomized so that the technician reading the LSI in the nephelometer did not know the origin of the samples.

The average reading for the fasting plasma sample was subtracted as blank from each sample reading (9). Means ±S.E.M. of the LSI were calculated. Statistical analyses, by Students *t*-test, of a-cs difference was carried out on the LSI. Where Intralipid® had been infused, LSI/l was converted into µmol exogenous triglycerides/l plasma using the standard curve and molecular weight of 878 for Intralipid® triglyceride. In the case of the oral fed state, the factor *K*, calculated from the following formula, was used to convert LSI/l into µmol/l.

$$K = \frac{TG_t - TG_0}{LS_t - LS_0}$$

here TG_0 is fasting arterial plasma triglyceride concentration, TG_t total plasma triglyceride concentration at time *t* after the meal, LS_0 the light scattering per unit volume of the plasma blank and LS_t the light scattering per unit volume of arterial plasma at time *t* after the meal. Storage of the diluted plasma samples for a few hours at room temperature before reading did not affect the results (10).

Electron microscopy

The arterial and coronary sinus blood samples were centrifuged for 10 min at 60 *g* and then 1% osmium tetroxide was added to the plasma (40). A drop of the fixed plasma was dried on Formvar-coated grids and these were then stained with uranyl acetate for 30 min at +60°C and

Table II *Arterial concentration and myocardial extraction of exogenous triglycerides—nephelometric determinations (means ± S.E.M.)*

a = arterial concentration, a-cs = arterial-coronary sinus difference, both in μmol/l plasma
Grand mean the mean of the individual means at 1 2, 3 and 4 hours in the I. fed state, or at 3 and 4 hours in the oral fed state

Subject no.	1 hour			2 hours			3 hours		
		a-cs	% of		a-cs	% of a	a	a-cs	% of
I.v. fed stat									
5	2 670 ± 10	220 ± 10*	8.2	2 720 ± 10	240 ± 20	8.8	2 120 ± 10	130 ± 20*	6.1
6	2 010 ± 20	170 ± 20	8.5	1 660 ± 10	20 ± 10 ns	1.2	1 840 ± 10	80 ± 10	4.3
7	3 150 ± 20	310 ± 30	9.8	3 270 ± 30	−30 ± 40 ns	−0.9	2 920 ± 30	90 ± 40	3.1
Mean ± S.E.M.	2 610 ± 330	230 ± 40	8.8 ± 0.5	2 550 ± 470	80 ± 80	3.0 ± 2.9	2 290 ± 320	100 ± 20	4.5 ± 0.9
Oral fed state									
8	390			1 290			1 570 ± 20	90 ± 30	5.7
9	900			1 310			1 590 ± 20	70 ± 20	4.5
10	320			320			900 ± 10	40 ± 20	4.4
Mean ± S.E.M.							1 340 ± 240	70 ± 10	4.9 ± 0.4

ns, $p > 0.05$ $p < 0.05$ $p < 0.01$ $p < 0.001$ (for individual observations based on *t*-test of difference between two means; for grand means based on *t*-test of paired differences).

thereafter with lead citrate for 3 min at room temperature. The samples were examined in a Siemens Elmiskop an. 1 Dr P. G. Lundqvist made and interpreted the electron microscopic part of the study

RESULTS

Exogenous plasma triglycerides

In the first series of studies, in which both chemical and nephelometric determinations of lycerides were made, eight observations of dif in triglyceride concentration across the coronary circulation (i.e. myocardial extraction of plasma triglyceride) were made in four subjects (Table I) Since determinations were in replicate, it has been possible to calculate the significance of each observation of myocardial extraction. Extraction of triglyceride measured chemically could have both an exogenous and an endogenous component, since total plasma triglyceride was measured. Seven of the eight chemical assessments showed a decrease in concentration across the coronary circulation (a positive a-cs difference) and of these seven three were significant. The nephelometric method should recognize particulate —in this case, presumably mainly exogenous— triglyceride. By this method six of the observations showed a significant positive myocardial extraction of plasma triglyceride and these six corresponded to positive a-cs differences obtained by the chemical method. One of the two negative a-cs differences found by the nephelometric method was significant, but it corresponded to a non-significant positive difference by the chemical method the other negative difference nephelometrically corresponded to a significant negative difference chemically The mean differences in μmol/l and the percentage differences obtained chemically and nephelometrically were quite similar There was a significant production of glycerol across the coronary circulation for all eight observations (Table I). This indicates that lipolysis had occurred in the coronary vasculature or in the myocardium during the i.v. infusion of fat emulsion. It is noteworthy that the two smallest productions of glycerol corresponded to negative nephelometric a-cs triglyceride differences.

Particles in plasma obtained from subject 3 (Table I) observed by electron microscopy were negatively stained and spherical. About 90% of these particles had diameters within the range 0.1–0.3 μ. When each of the four arterial specimens was compared with its corresponding coronary sinus specimen no difference in diameter could be detected However an exact calculation of mean diameter was not possible.

The details of one oral and one i.v. fed state study the first studies of the second and third series, are shown in Fig. 2.

In the three subjects of the second series of studies, in which fat emulsion was infused i.v. a

4 hours			Grand mean ± S.E.M.	
	a-cs	% of		a-cs
2 810 ± 10	390 ± 10*	13.9		
2 130 ± 10	100 ± 30	4.7		
2 680 ± 30	−40 ± 30 ns	−1.5		
2 540 ± 210	150 ± 30	5.7 ± 4.5	2 500 ± 150	140 ± 40
780 ± 20	150 ± 30*	19.2		
1 500 ± 20	90 ± 30*	6.0		
860 ± 20	−30 ± 40 ns	−3.5		
1 050 ± 230	70 ± 50	7.2 ± 6.6	1 190 ± 160	70 ± 20*

relatively steady state over 4 hours with respect to the arterial concentration of fat particles was achieved for the 4 hours of infusion (Table II). Nine out of twelve nephelometric observations of myocardial extraction of plasma triglyceride were significantly positive. The grand mean extraction during the i.v. fed state was significant at the $p < 0.01$ level.

During alimentary lipaemia, the third series of studies, five out of six nephelometric observations of myocardial extraction of plasma triglyceride were significantly positive (Table II). The grand mean extraction (3 and 4 hours) was also significant at the $p < 0.05$ level. None of the significant observations of myocardial extraction of plasma triglyceride in either the oral or the i.v. fed state can be accounted for by shifts in plasma water as shown by a comparison of the percentage changes in triglyceride and ^{125}I-albumin (Tables II and III). However, each negative a-cs difference in triglyceride concentration, albeit non-significant, is associated with a similar change in ^{125}I albumin.

Glycerol

There were significant productions of glycerol across the coronary circulation of the six observations during the i.v. fed state and at each of the six made during alimentary lipaemia (Table IV). In the case of the i.v. fed state there were also consistent rises (about threefold) in the arterial concentrations of glycerol from preinfusion values.

Arterial FFA concentrations

Arterial FFA concentrations showed a tendency to rise in the i.v. and oral fed states, but not to more than twice fasting concentrations (Table V). Significant myocardial extractions of FFA were observed for all six observations during the i. fed state and on five out of six occasions in the oral fed state in which a sufficient number of determinations was made to warrant statistical evaluation.

Arterial blood glucose concentrations

During the i.v. infusions of fat emulsion and glucose solution, blood glucose concentrations were about double the fasting concentrations; three out of six observations of myocardial extraction of glucose were significant (Table VI).

Following the fat-rich meal, on the other hand, arterial blood glucose concentrations showed little change from fasting concentrations (Table VI). Furthermore, only one of the six observations of glucose extraction was significant.

Oxygen saturation

The oxygen saturation of arterial and coronary sinus blood during alimentary lipaemia and the i.v. fed state are shown in Table VII.

DISCUSSION

Methodology

The micronephelometer (47) used in the present investigation has been shown to give readings well correlated with plasma triglyceride concentration (23 31 43, 44). The method is also especially sensitive in the recognition of larger particles, since increase of the particle radius by a factor is equivalent to increasing the LSI by at least the square of that factor (47). After a fat-rich meal nearly 80% of the LSI has been shown to be due to particles of chylomicron size, with a diameter greater than 0.1 μ (43). Fat particles in Intralipid® S had diameters up to 1 μ with most particles in the range 0.1–0.3 μ. Direct comparison of determination of exogenous triglycerides in plasma, during removal of Intralipid® after i. injection, by a chemical method (involving PVP gradient separation of exogenous triglycerides followed by estimation of glyceride-glycerol) and by nephelometry showed excellent agreement between

Table III. *Arterial radioactivity and a-cs difference in radioactivity of ¹³¹I-albumin (cpm/0.5 ml plasma)*

Subject no.	2 hours			3 hours			4 hours		
	a	a-cs	% of a	a	a-cs	% of a	a	a-cs	% of a
I.v. fed state									
5	114.5±0.7	0.2±1.1 ns	0.2				102.9±0.4	−1.4±1.0 ns	−1.4
6	67.2±0.8	−0.8±1.3 ns	−1.2				62.6±0.5	0±0.8 ns	0
7	167.4±1.2	−3.9±1.7 ns	−2.3				164.2±2.4	−4.0±.4 ns	−2.4
Mean	116±29	−1.5±1.2	−1.1±0.7				90±14	−1.8±1.2	−1.3±0.7
Oral fed state									
8				123.0±1.3	0.5±2.0 ns	0.4	117.0±1.4	1.4±1.8 ns	1.2
9				171.9±0.7	0±1.0 ns	0	173.4±1.7	2.0±2.3 ns	1.2
10				283.2±1.8	0.9±2.1 ns	0.3	267.9±1.0	−9.1±1.6	−3.4
Mean				193±47	0.5±0.3	0.2±0.1	186±44	−1.9±3.6	−0.3±1.5

Symbols and abbreviations as in Table II.

the two methods (31). Microenphelometry therefore, seemed applicable to the problem of measuring differences in concentration of triglyceride between arterial and coronary sinus blood in the oral and i.v. fed state. As discussed in detail elsewhere (8), a method with high precision is required for determination of arterio-venous differences of triglycerides. Thus a method involving separation of exogenous from endogenous triglyceride (6) prior to triglyceride determination would certainly be accompanied by a methodological error too large to allow statistically significant concentration differences to be observed.

At the same time micronephelometry has theoretical limitations. If the spectrum of particle size in arterial blood during the i.v. infusion of a fat emulsion was the same as that in the emulsion, then, to use the emulsion as a standard, as was done, would be to give a reliable triglyceride concentration of arterial plasma particles. However it is known that, following a fat-rich meal and after infusion of Intralipid® some increase in plasma content of smaller secondary particles occurs with time (43). Although these particles are smaller they will contribute slightly to the light scattering of plasma (31), so that to subtract the LSI of the fasting plasma blank from readings during infusion to obtain the concentration of exogenous triglycerides is an approximation. Another theoretical objection is that, on passage

Table IV. *Arterial concentration and a-cs difference in concentration of glycerol*

Subject no.	0	2 hours		3 hours		4 hours		Grand mean ± S.E.M.	
		a	a-cs	a	a-cs		a-cs		a-cs
I.v. fed state									
5	72±0	225±1	−27±2			223±1	−22±2		
6	33±0	104±2	−12±3			60±0	−20±2		
7	43±1	1.4±1	−20±1			167±2	−22±3		
Mean±S.E.M.	49±12	151±37	−20±4			150±48	−21±1	151±27	−21±2
Oral fed state									
8	91±1			87±2	−33±3	105±1	−19±1		
9	22±1			4±1	−13±2	53±1	− 8±1		
10	52±1			55±1	− 5±1	35±1	−.4±2		
Mean±S.E.M.	55±20			42±23	−17±8	64±21	−17±5	53±15	−17±4

Symbols, abbreviations and units as in Table II.
Grand means do not include preinfusion (0) values.

through the coronary circulation, larger particles might be transformed into smaller particles without a change in concentration of plasma triglyceride. Thus LSI could decrease without a change in triglyceride concentration. The electron microscopy screening of particle size on four occasions in one subject (no. 3) did not reveal any major change in diameter across the coronary circulation, although there was a significant reduction of LSI on all occasions. To test this possibility further the comparison between the chemical and nephelometric methods described in Table I was performed. The correlation between the two methods (r between the individual values was 0.68) and the closeness of the grand means for myocardial triglyceride uptake by the two methods suggest that the decrease in LSI is a direct quantitative estimate of a decrease in exogenous triglyceride concentration.

In the case of alimentary lipaemia a suitable standard for converting LSI to triglyceride concentration was not available. We therefore measured total plasma triglyceride and also LSI for endogenous plasma triglyceride and after the fat meal. The change in total triglyceride was equated with the change in LSI. This conversion of LSI to triglyceride will slightly overestimate the exogenous triglyceride, as the rise in total triglyceride not only comprises the exogenous triglyceride but also some endogenous triglyceride (6). This will mean that the a-cs concentration difference will be overestimated. However if the same conversion factor can be applied to arterial LSI and to the a-cs difference in the LSI, the percentage change is unaffected by such an assumption.

The chemical method for estimating a-cs differences in total triglyceride concentration and the nephelometric method for estimating a-cs differences in, essentially exogenous triglyceride concentration were in substantial agreement in the first series. It is possible that little myocardial extraction of endogenous plasma triglyceride, known to take place in the fasting state in man (8), takes place in the presence of exogenous triglyceride. At high plasma triglyceride concentrations, as in lipaemia of exogenous origin given a certain analytical error it is more difficult to recognize a given a-cs difference than at low concentrations. This is, perhaps, one reason why significant a-cs differences were seen less often with the chemical than with the nephelometric method.

Table V *Arterial concentration and myocardial extraction of FFA*

Subject no.	0	1 hour	a-cs	2 hours	a-cs	3 hours	a-cs	4 hours	a-cs	Grand mean ±S.E.M. a	Grand mean ±S.E.M. a-cs
I fed state											
5	710	960±20	140±20	1 370±10	120±10	860±20	130±30	840±10	70±10		
6	380	1 200±30	40±40	760±10	60±20	630±20	90±20	580±0	100±10		
7	560	590±20	100±20	620±10	30±10	770±10	60±20	740±10	130±10		
Mean ± S.E.M.	560±100	920±180	90±20	920±230	70±30	750±70	90±20	720±80	100±20	830±70	90±10
Oral fed state											
8	980	1 070	—	1 320	—	1 320±20	170±20*	1 050±10	−40±20 ns		
9	600	800	—	910	—	1 140±10	250±10*	1 170±0	200±10*		
10	930	860	—	960	—	940±20	90±20*	1 030±10	80±10*		
Mean ± S.E.M.	840±120	910±80	—	1 060±130	—	1 070±70	170±50	1 090±40	80±70	1110±50	130±40

Symbols, abbreviations and units as in Table II.
Grand means do not include preinfusion (0) arterial concentrations or arterial concentrations where there is no corresponding coronary sinus concentration.

Table VI. *Arterial concentration and myocardial extraction of glucose (µmol/l whole blood)*

Subject no.	0	1 hour		2 hours		3 hours		4 hours		Grand mean ±S.E.M.	
			a-cs		a-cs	a	a-cs		a-cs		a-cs
Iv fed state											
5	4 600	9 170	—	9 900±60	100±70 ns	9 410	—	7 900±40	70±60 ns		
6	5 070	8 620	—	7 570±80	150±100 ns	7 670	—	7 650±40	180±60		
7	4 730	9 100	—	9 060±80	530±110	7 530	—	8 580±70	710±90		
Mean ± S.E.M.	4 800±40	8 960±180		8 840±680	260±40	8 200±610		8 400±270	320±200	8 440±370	290±110 ns
Oral fed state											
8	4 320	4 410	—	4 450	—	4 430±30	110±50 ns	5 170±30	30±40 ns		
9	5 000	4 870	—	5 030	—	4 460±60	70±90 ns	4 760±80	180±100 ns		
10	4 910	3 380	—	4 500	—	4 220±50	170±70*	4 510±70	30±90 ns		
Mean ± S.E.M.	4 790±210	4 220±440		4 660±190		4 370±80	70±70	4 810±190	80±50	4 590±140	80±40 ns

Symbols and abbreviations as in Table II.
Grand means do not include preinfusion (0) arterial concentrations or arterial concentrations where there is no corresponding coronary sinus concentration.

Myocardial removal of exogenous plasma triglyceride

In both alimentary lipaemia and the Iv fed state, using the nephelometric method, exogenous plasma triglyceride concentrations decreased by grand means of 6% across the coronary circulation. In the fasting state in man the myocardial extraction of plasma triglyceride is about 1.5% or if very low density lipoprotein (VLDL) is the active triglyceride fraction, about 3% of that fraction (8). The twofold difference in myocardial extraction of exogenous and VLDL plasma triglyceride is very similar to the two- to threefold difference in turnover times of chylomicra and Intralipid® on one hand and VLDL on the other (4, 16).

In the fasted experimental animal myocardial lipoprotein lipase activity is higher than in the fed state (21, 49). The implication has therefore been that myocardial removal of exogenous triglycerides, only available physiologically after a fat meal, might be of little physiological importance. Our data show however that there is a continuous uptake of triglycerides by the heart during feeding.

The observations 3 and 4 hours after a fatty meal were made at about the peak of fat absorption and when blood glucose concentration would be falling after a mixed meal. It might be argued that, were glucose available as an alternative substrate, it would be extracted by the myocardium in preference to exogenous triglyceride. In the

Table VII. *Mean blood gas values (ranges within parentheses)*
S = saturation, C = content

	2 hours			3 hours			4 hours		
	S_{O_2} (%)		$C_{(a-cs)O_2}$ (ml/l)	S_{O_2} (%)		$C_{(a-cs)O_2}$ (ml/l)	S_{O_2} (%)		$C_{(a-cs)O_2}$ (ml/l)
		cs			cs				
Iv fed	96.2 (95.2—97.3)	34.3 (33.0–42.4)	108.7 (97.4–117.8)	—	—	—	97.7 (97.3–98.1)	39.6 (32.6–48.1)	107.4 (96.6–119.2)
Oral fed	—	—	—	96.4 (96.0–96.8)	29.1 (24.8–32.5)	121.1 (111.7–127.5)	96.5 (95.7–97.1)	30.6 (26.9–38.5)	119.3 (106.2–130.2)

isolated perfused rat heart this is not the case (42). Nor was it in the present investigation in the i.v. fed state, where arterial blood glucose concentrations were nearly double fasting concentrations and yet myocardial extraction of exogenous plasma triglyceride was as demonstrable as in the oral fed state.

The mean (±S.E.M.) myocardial oxygen extraction ratios (OER, i.e. the fraction of the a-cs difference in oxygen content which could be accounted for by complete oxidation of a particular substrate) for triglyceride were 51±14 and 67±32 in oral and i.v. fed states, respectively. In the oral fed state OERs for FFA and glucose were 31±7 and 10±4, respectively. Thus, since the sum of OERs for the main substrates is close to 100%, they may have been utilized immediately. However, in the i.v. fed state, where OERs for FFA and glucose were 25±5 and 37±14 and the sum of OERs 129, this has probably not been the case. Part may be stored in the myocardium as triglyceride or glycogen. It is noteworthy here that the a-cs oxygen differences observed in the oral and i.v. fed states are similar to those observed previously in the fasting state (28); there is disagreement about this point when the isolated perfused rat heart is perfused with and without chylomicra (13, 34, 38).

In earlier studies in resting fasting man (28) no significant glycerol production across the coronary circulation was observed. In rats injected with ^{14}C-glycerol and ^{3}H-palmitic acid labelled chyle, the hearts had a $^{14}C/^{3}H$ ratio much lower than the injected chyle (2). Similar results have been found in rabbit hearts (17). This means that plasma triglyceride is lipolysed prior to uptake of its fatty acid, and release of glycerol into the coronary vascular bed might be an index of such lipolysis. The only circumstance in which there is evidence of an intramyocardial source of glycerol in coronary blood in man is during prolonged exercise (24). Every observation in the oral and i.v. fed states showed a significant glycerol release into the coronary vascular bed. The following quantitative interpretation concerning lipolysis (glycerol release) and triglyceride uptake can be made. First we assume that the glycerol release only derives from exogenous plasma triglycerides and that the change in LSI directly relates to disappearance of exogenous plasma triglycerides. The average glycerol release of about 20 µmol/l plasma (both oral and i.v. fed state) would correspond to about 19 µmol/l blood (45). The average triglyceride disappearance was around 40 and 80 µmol/l blood of triglyceride-glycerol in the oral and i.v. fed state, respectively. The minimum figure for total hydrolysis of exogenous plasma triglycerides to fatty acids and free glycerol would thus be 25 and 50%, respectively. This means either that extraction of triglyceride has been overestimated or glycerol production underestimated, or that uptake of intact or partially hydrolysed triglycerides takes place, or that glycerol produced on lipolysis is extracted. That the last mentioned alternative is possible is supported by the demonstration of glycerol kinase activity in the rat heart (36). However, the increases in plasma glycerol concentration, relatively greater than the increase in plasma FFA concentration, during the i.v. fed state, must reflect lipolysis of fat emulsion in tissues, possibly including the myocardium, where glycerol is less readily used than FFA. The amounts of glycerol and FFA in the infused emulsion cannot account for the increases in their plasma concentrations (see Methods).

Myocardial extraction of plasma FFA and glucose

There is less myocardial net extraction of plasma FFA in the present fed state than in our previous fasting studies (28). This could mean that not all FFA released on lipolysis of exogenous triglyceride are taken up by the myocardium, some entering coronary venous blood, or that there is a competition between the two sources of fatty acids for uptake by the myocardium.

The numerical values of myocardial extractions of glucose during alimentary lipaemia were, in spite of similar blood glucose concentrations, about half those found in the fasting state and were significant in only one out of six observations (28). In the isolated perfused rat heart, chylomicra reduce glucose uptake (34). It has recently been demonstrated in man that the myocardial extraction of blood glucose is inversely related to that of plasma FFA (28, 29). The present findings indicate that FFA arising from lipolysis of chylomicra in the coronary vascular bed might reduce myocardial extraction of blood glucose. In the i.v. fed state blood glucose concentrations were considerably elevated over fasting concentrations.

Thus, with the same percentage analytical error it should be more difficult to detect significant a-cs differences unless the fractional extraction is the same. This partly explains why even though numerically the grand mean glucose extraction in the fed state lies above that found in the fasting state (28), it is non-significant. In one of the three subjects (no. 7), however both observations of glucose extraction were significant and high. The grand mean percentage glucose extraction (3%) in the fed state was of the same order as, or slightly lower than, that found in the fasting state (4%) (28).

CONCLUSIONS

This investigation suggests that exogenous plasma triglycerides can be lipolysed on passage through the coronary circulation. The measured disappearance of exogenous triglyceride is sufficient to provide an important source of energy for myocardial metabolism, although it cannot be stated whether the triglyceride fatty acid extracted by the myocardium in the fed state is used directly or whether it is stored. It is possible that other sources of myocardial fuel, blood glucose and plasma FFA, are not as important during alimentary lipaemia as they are in the fasting state.

ACKNOWLEDGEMENTS

This work was supported by grant from the Swedish Medical Research Foundation (No. 19x-204). M. L. W. was recipient of Life Insurance Medical Research Fund of Australia and New Zealand Overseas Research Fellowship.

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Acta med. scand. Vol. 193 pp. 247–249 1973

ACUTE MYOCARDIAL INFARCTION OCCURRING DURING CONTINUOUS ECG RECORDING

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Abstract. Ten hours after admission to a coronary care unit because of accelerating angina on effort a 60-year-old woman experienced prolonged chest pain. S-T segment elevations, sinus and nodal bradycardia and ventricular tachyarrhythmias occurred within minutes, and pathologic Q waves appeared within one hour. Serial transaminase estimations and ECGs confirmed the development of transmural anterior myocardial infarction. Cases like this may fill the gap in our knowledge about the earliest phase of myocardial infarction in man.

In recent years the knowledge of the clinical features in acute myocardial infarction (AMI) has increased considerably mainly from intensified observation in specialized coronary care units (CCU). However the delay between onset of symptoms and admission to these units result in a lack of knowledge of the first stages of AMI. To shorten this delay Pantridge and Geddes (6) organized a mobile CCU and experiences from such units have added to our knowledge of the early phases. The remaining gap in our knowledge may be filled by experience from patients developing infarction after their admission to a CCU because of accelerating angina.

METHODS

At the CCU of Serafimerlasarettet the ECG is monitored on a memory oscilloscope and recorded continuously with an ink writing electrocardiograph (Mingograf 81, Elema-Schönander, Solna, Sweden) at 10 mm/sec. Admission criteria include accelerating angina. Other details about the CCU, its policy and criteria have earlier been presented (4).

CASE REPORT

A 60-year-old woman with bilateral ovarectomy at the age of 41 and a 10-year history of hypertension was admitted because of accelerating angina on effort since one week. On admission she was in good condition with normal physical findings from heart and lungs, and a 12-lead ECG did not show any signs of acute myocardial infarction (Fig. 1 A).

During 10 hours in the CCU she had no pain. She had a regular sinus rhythm between 60 and 80 beats/min, and the arterial BP varied between 160/90 and 180/100 mmHg. Her respiratory rate was 18/min. As routine prophylaxis she was given a continuous infusion of lignocaine at a rate of 2 mg/min. After 10 hours she experienced for the first time angina at rest, and it was more intense than ever before. Her arterial BP abruptly fell to 110/80 and her heart rate to about 40 (Fig. 2). Within 2 min following the drop in heart rate S-T segment elevations appeared on the continuous ECG. The pain persisted, and a 12-lead ECG taken 10 min later showed S-T segment elevations compatible with acute anterior infarction (Fig. 1 B). No pathologic Q waves were visible but in further recordings 30 min and 2 1/2 hours later they were fully established, confirming the development of transmural anterior infarction (Fig. 1 C, D).

The bradycardia was initially sinus, later nodal in origin. Five minutes after its onset methyl scopolamine, 0.5 mg, was given i.v. according to a marking on the continuous ECG. Three minutes after the injection the heart rate had increased from 40 to about 100/min. In spite of the prophylactic lignocaine infusion ventricular ectopic beats appeared within 3 min following the onset of bradycardia; at first only as single beats, later also in salvos and of multiform appearance as well as of the R-on-T type. A first bout of ventricular tachycardia, i.e. 3 or more consecutive ventricular ectopic beats, appeared 7 min after the drop in heart rate, and occurred repeatedly during the following 30 min. About 75 min later, following further therapy (Fig. 3), these arrhythmias had disappeared with the exception of occasional ventricular ectopic beats, and no other arrhythmias had occurred. The pain continued in spite of analgesics. The arterial BP was 150/100, and occasional rales were auscultated. Serial transaminase estimation revealed a peak value of 290 units for S-GOT 26 hours after onset of pain; 62 units for S-GPT and 2390 units for S-LDH. The patient left the hospital in good condition after 28 days.

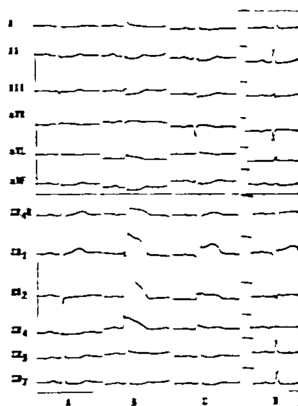


Fig. 1. Four 12-lead ECGs, recorded on admission (A), and after about 10 min (B), 40 min (C) and 3 hours (D) from onset of chest pain in the CCU.

DISCUSSION

The clinical course and the ECG development support the assumption that an anterior transmural infarction occurred during the patient's stay in the CCU. Following one week of accelerating angina on effort she had in the CCU her first attack of anginal pain at rest, 10 hours after admission, and it was also her first one lasting for several hours. Within minutes S-T segment elevations appeared, and $^1/_2$ hour later pathologic Q waves as well were present.

Unfortunately the onset of pain was not marked on the continuous ECG but could be estimated to have preceded the S-T elevations only by a few minutes. Studies on experimental myocardial infarction have shown the S-T segment elevations to occur $^1/_2$–3 min after coronary occlusion (3, 7).

In this case sinus bradycardia appeared about _ min following the estimated time of myocardial injury, the first ventricular ectopic beats about 3 min later and the first ventricular tachycardia after about 7 min. The sinus and later nodal bradycardias were treated and abolished within a few minutes, so it could not be known how long they would have persisted. Adgey et al. (2) found in 19 patients with an acute anterior infarction, admitted within 30 min after the onset of symptoms, not a single case of bradycardia, in con-

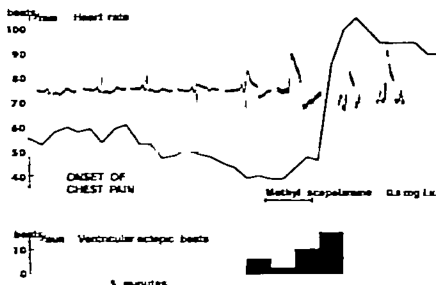


Fig. 2. Development of S-T segment elevation and arrhythmias within 15 min from onset of chest pain.

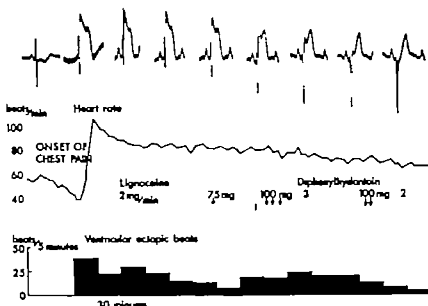


Fig. 3 Development of pathologic Q wave and arrhythmias within 75 min following onset of chest pain. The ventricular ectopic beats were often multiform, of the R-on-T type or occurring in salvoes during the first hour.

trast to 5 out of 15 with posterior myocardial infarction. Even if the spontaneous course of this initial bradycardia would be short, it may be important because of the association, as in this case, with a marked fall in arterial BP. Recently Stock (8) has stressed that cardiac slowing and not cardiac irritability may be the major problem in the earliest phase of myocardial infarction.

The first ventricular ectopic beats occurred before the injection of methyl scopolamine, but the first ventricular tachycardia not until 2 min following it, and consequently it may possibly have been provoked by this drug as earlier described (5). Ventricular ectopic beats have been observed in 70 patients, ventricular fibrillation in 28 and ventricular tachycardia in 10 patients out of 284 monitored within one hour from onset of symptoms (1). The spontaneous course of the ventricular tachyarrhythmias in this case was probably influenced by the routine constant rate lignocaine infusion which was in progress when the infarction occurred, and later by additional diphenylhydantoin therapy.

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Acta med. scand. Vol. 193 pp. 251–255 1973

THE EARLIEST PHASE OF ACUTE MYOCARDIAL INFARCTION IN MAN

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Abstract. The events in the earliest phase of acute myocardial infarction (AMI) in man are relatively unknown. This is case report of patient already treated in a coronary care unit and monitored on ECG when developing an AMI. A very early appearing bradyarrhythmia was noted and its significance is discussed in connection with possible mechanisms of sudden death.

Much has been learned from animal experiments regarding the initial phase of acute myocardial infarction (AMI). These experiments cannot, however be a substitute for man himself and can only function as a clue to what may happen in the human being during the earliest phase of an AMI. Coronary care units (CCU) have focused their main interest on arrhythmias and much knowledge has been gained about the different types of arrhythmias, their significance and their treatment. One of the major problems that remain to be solved is to shorten the delay from the onset of symptoms to the admission to a CCU since the highest mortality in AMI is found in the earliest stages. Also, because of this delay little is known about the very first events of an AMI in man. The ideal situation for studying the earliest period in AMI is when patients are already in a CCU monitored on ECG, when they start to develop the myocardial necrosis. These cases are rare and therefore merit description, as they may form the basis for possible antiarrhythmic treatment administered prior to admission to hospital.

METHODS

The patient reported was treated in the CCU at Serafimerlasarettet. Admission and diagnostic criteria as well as routines hav been described elsewhere (13). The ECG was monitored on memory oscilloscope and recorded on paper with an ink-writing electrocardiograph at 10 mm/sec. Enzymes, i.e. SGOT SGPT SLDH and the heat stable portion of SLDH, ere analysed on admission and twice daily at 12-hour intervals. Diagnostic 12 lead ECGs were recorded on admission and at least once daily At autopsy the heart was examined by serial transverse-slice method (11) and the slices were stained with nitro-BT (9 12). The infarct size was estimated using point counting technique (6) and expressed in % of the left and right ventricular masses, respectively

CASE REPORT

Case history

The patient was 71 year-old woman with family history of diabetes and death at an early age. In 1937 she had pleurisy due to tuberculosis and in 1949 right-sided nephrectomy was performed because of renal tuberculosis. About 2 years before her present illness the patient experienced occasional anginal pain, particularly when walking in cold air. The patient used nitroglycerine in small amounts.

A normal 12-lead ECG was recorded in Feb. 1972, but an exercise test showed pathological ST T depressions in the precordial leads after 5 min work at 400 kpm/sec consistent with coronary insufficiency Casual BP 190/90. Serum cholesterol 275 mg% Triglycerides 100 mg% I. glucose tolerance test normal. X-ray showed normal heart size and lung fields.

On Aug. 21, 1972, the patient experienced increased pain during walking but no other symptoms, and on the following day she took walk without any pain On the morning of Aug. 24 she felt uneasy and experienced chest pain indoors and had to rest in chair. She sought medical help and was admitted to the CCU because of progressive angina pectoris. Physical examination revealed nothing abnormal. BP 190/90; 12-lead ECG was unchanged compared to the one recorded 6 months before, and the enzymes ere normal. Anticoagulant treatment was started with dicoumarol on the day of admission. At 10.27 a.m. on the following day the patient developed severe chest pain during defaecation.

Arrhythmias

An ECG recorded 2 min before the onset of chest showed sinus rhythm with rate of 65/min.



Fig. 7. Transverse heart slice stained with nitro-BT showing recent infarction in the lateral and inferior left ventricular wall, including both papillary muscles and also engaging the posterior septum and the inferior right ventricular wall.

reflecting right heart failure and liver cell necrosis. Serum creatinine increased from 1.3 mg% on the 25th to 6.2 mg% on the 29th and potassium from 3.6 mEq/l to 6.3 mEq/l. The PP value was 95 on admission, 56 on the following day, 14 on Aug. 26, and around 10 on the following days.

Autopsy

The heart weighed 355 g. A fresh fibrinous pericarditis was found. A stenosis with an ulcerated plaque was found in the left circumflex artery 35 mm from the aorta and a 20 mm long thrombus was present proximally and distally to this stenosis. A transmural infarction was found, involving 55% of the left ventricular mass and located in the inferior and lateral left ventricular wall, also engaging the posterior part of the septum. Both papillary muscles, part of the right inferior ventricular wall and 15% of the right ventricular mass, were also infarcted (Fig. 7). The left circumflex artery was dominant and the right coronary artery hypoplastic. In the left pleural space adhesions from an old pleuritis were found. In the right pleural space 1 000 ml of fluid was present. The lungs were slightly oedematous. The liver was congested, as was also the left kidney. Microscopically a fresh infarction with an age of a little less than one week was found and in the kidney there were signs of acute tubular necrosis.

DISCUSSION

This case brings up some interesting observations. The first is the very early, almost instantaneously appearing bradycardia. Studies by the Belfast group have shown bradycardia to be a common event in AMI during the first 60 min (1, 10).

This very early bradycardia, which was also described in the case of Mogensen and Orinius (8), is of great interest as regards possible mechanisms of sudden death, since bradycardia might increase the risk of lethal arrhythmias (4). This does not mean that all cases of sudden death will develop an AMI if resuscitated, and we have seen patients who, after resuscitation showed no clinical signs of AMI.

Recent experiments in the cat by Thorén (14) have shown that occlusion of a coronary artery starts an increased activity of cardiac receptors within the left ventricle, leading to bradycardia and hypotension within 1 min due to increased vagal efferent discharge. These afferent impulses are also set off by anoxia or distension of the left ventricle.

The early bradycardia in our case fits in well with the idea of a vagal reflex mechanism as proposed by Thorén (14). It may not only be the occlusion that is of importance, but a sudden change in the myocardial circulation leading to anoxia might also increase the nervous activity since many cases of AMI in man have no demonstrable coronary artery occlusions at all (2) and the thrombus may be a secondary event (5). The very early appearance of bradycardia is also of interest, since this means that there might be a fundamental difference between angina pectoris and some cases of AMI already in the first minutes after the onset of pain, and the generally entertained idea that angina pectoris is probable when the pain is shorter than 15 min might not be the only practical difference. A slow pulse appearing very early after the onset of chest pain might favour the diagnosis of AMI compared to other causes of chest pain.

From Thorén's cat experiments it was also learned that the receptors within the left ventricle could be blocked by lignocaine. This is of interest since there has been some controversy regarding administration of lignocaine in AMI when bradycardia is present (3). Lignocaine given very early in the course of AMI might in fact prevent both the bradyarrhythmias and the potentially lethal ventricular arrhythmias.

The second interesting observation is the time of appearance of the ventricular ectopic activity. The first appearance of ectopic ventricular activity in our case was seen after approximately 40 min, an interval that is sufficient to cause irreversible damage to myocardial cells and to start necrosis (7). Ventricular ectopic activity in the case of Mogensen and Orinius (8) started much earlier, already 4 min after the onset of symptoms. Their

first ventricular tachycardia appeared later and the authors speculate on the possibility of methyl scopolamine, which had been given, as a provoking agent. Their patient was also on i.v. lignocaine. In our case no ventricular ectopic activity was noted closely following on the injection of methyl scopolamine.

The death of our patient was inevitable as far as can be judged and was caused by an extensive myocardial infarction leading to progressive heart and renal failure. It is insufficiently known today *and renal failure. If the development observed in* this patient reflects the initial stages of what would lead to a sudden death in a normal environmental setting, one may speculate about the possible effects of actions taken to prevent such deaths. More must therefore be learnt about the mechanisms of sudden death and the described patient might contribute to this knowledge.

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Congress Announcements

The Tenth Congress of the European Dialysis and Transplant Association will be held in Vienna, Austria, June 28–30 1973

Secretariat Wiener Medizinische Akademie, Frl. E. Maurer Alser Strasse 4 A 1090 Vienna, Austria.

IAEA Symposium on Radioimmunoassay and Related Procedures in Clinical Medicine and Research will be held in Istanbul, Turkey Sept. 10–14 1973 Further information, participation forms and forms for submission of a paper intended for presentation at the Symposium will be obtainable from national authorities for atomic energy matters. Abstracts of such papers must be submitted to the International Atomic Energy Agency through these authorities.

Organizers. International Atomic Energy Agency Kärntner Ring 11–13 A 1010 Vienna, Austria.

Scientific Secretaries: Dr E. J Garcia and Dr E. H. Belcher Medical Applications Section.

The Third International Symposium on Atherosclerosis will be held in Kongresshalle, West Berlin Germany Oct. 25–28 1973.

Correspondence Kongressgesellschaft für ärztliche Fortbildung e.V D 1 Berlin 41 Wrangelstraße 11–12. West Germany

Deadline for submission of abstracts. postmarked April 14 1973.

Acta med. scand. Vol. 193 pp. 257–258, 1973

Editorial

FROM BAD TO WORSE?

Treatment and Leukemogenesis

It has long been known that patients with polycythemia vera (PV) who have been treated with ^{32}P may develop acute blast cell leukemia. Interestingly enough this has been interpreted in different ways. One very extensive and careful statistical analysis has led to the conclusion that the leukemia is the result of the ionizing radiation. Another critical study concerns the development of acute myeloic leukemia (AML) as part of the "natural" history of PV. These authors maintain that a patient who lives long enough runs a considerable risk of dying from this "complication" and that patients live longer when treated with ^{32}P. It has been maintained that we should treat our patients with Myleran® instead, but this is perhaps not a logical conclusion as a number of patients with chronic myeloic leukemia (CML), who respond well initially to such treatment, finally develop AML and die.

There are a few facts that are relevant in this connection. After treatment with ^{32}P for PV and with Myleran® for CML, acute blast cell leukemia is not rare. Patients, who were treated in Great Britain for ankylosing spondylarthritis with X-rays to the spine developed significantly more AML (but never acute lymphatic leukemia) than untreated normals. Among the survivors of the atomic bomb in Hiroshima the same result was established—only an increase in myeloic leukemia was observed.

During the last years a number of observations have been made in patients with myeloma who were treated with cytostatic drugs, chiefly with Melphalan® and developed AML (so-called monomyeloblastic). The first was the already classical case of Osserman with maximal lysozymuria and monocytic leukemic proliferation (6). A great number (20–25) of such cases are now known. It had not been observed previously that myeloma cases develop AML even if it may be possible that some instances of so-called plasma cell leukemia were in reality blast cell leukemias. It seems difficult to imagine why a plasmocytoma should develop into a blast cell leukemia of monocytic type. Still more far-fetched is this explanation in a number of recent observations. Catovsky and Galton (2) have described a case of chronic lymphocytic leukemia (CLL) treated with chlorambucil for 40 months. He then developed unquestionable monomyeloblastic leukemia with maximal lysozymuria and died. It is well established that lymphocytes do not produce lysozyme, and acute lymphoblastic anemia after CLL is extremely rare—many say non-existent. That this patient switched over from a lymphocytic proliferation or had a superimposed monomyeloblastic malignancy is therefore remarkable. There are now a number of other observations of similar kind. Macroglobulinemia (chlorambucil or Melphalan®) into blast cell leukemia or erythroleukemia is another lymphatic disease with this complication. Also patients with carcinoma (from lung or ovary) have died from acute leukemia after treatment with only Thiotepa® and no irradiation. Some patients have also been treated with cyclophosphamide, but they were also exposed to radiation and the effect of the drug is therefore difficult to assess (3).

In summary it may be said that a number of therapeutic agents—ionizing radiation (X rays, ^{32}P), Melphalan® chlorambucil, busulphan, Thiotepa®—have been used for a considerable time in patients who have developed acute leukemia later

An interesting paper in the present issue of this journal treats the question of cytogenetic studies in conditions where we know that acute blast cell leukemia occurs with increased frequency as a final event. The reader will find much food for thought in this paper. One of the great difficulties in such studies is the fact that we do not know how long the preleukemia period has lasted before we have enough symptoms to make a clinical diagnosis. There is, however, one very diagnostic "marker" for myeloic leukemia in the karyotypic picture. This is a small extra chromosome split off from the normal 22 according to the latest investigations (the reader may consult Caspersson's excellent review of these subjects). This extra chromosome is called the Philadelphia chromosome and patients who carry it are said to be Ph. Recent work also on myeloma indicates that there is a premyeloma stage with only γ-globulin changes before visible myeloma comes to the surface. A recent number of the Lancet contains a very interesting study (1) regarding this "subterraneous" existence of leukemia before it emerges with clearcut symptoms. A patient was followed for over 5 years with a low percentage of the Philadelphia chromosome in his bone marrow cells. On some occasions he had slight leukocytosis but otherwise no symptoms of blood disease until he suddenly fell ill with blast cell leukemia. At this stage the marrow contained 90% Ph cells. These facts are interesting in themselves, but still more important is the finding that another person had a mosaic of two different karyotypes XXY and XY (normal). This means that two different cell lines could be distinguished and the question arose whether the Ph chromosome was present only in one of them. As a matter of fact it was only found in the normal XY cells. This must mean that non-leukemic and leukemic cells may exist together in the same organism (5). Whether or not this has a bearing on Fialkow's findings that normal donor cells became leukemic on transplantation into a leukemic recipient (4) cannot be discussed here. This is an interesting problem of great practical importance when judging the feasibility of transplanting normal marrow to leukemic subjects.

The problem of malignancy after irradiation and after administration of cytostatic drugs has both practical and theoretical applications in another field. Persons with transplanted organs, who are under immunosuppression with antimetabolites—usually Imurel® (azathioprine)—suffer a certain risk in this respect. It is possible that the development of malignant lymphoma, usually reticulum cell sarcoma, in these cases is more related to immunosuppression than to active "lymphomogenesis".

Only continued observations will give an answer to the problem.

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Jan G. Waldenström

Acta med. scand. Vol. 193, pp. 259–266, 1973

JAUNDICE IN PREGNANCY

A Follow-up Study of the Series of Women Originally Reported by L. Thorling

I. *The Pregnancies*

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Abstract. A monograph by the late L. Thorling entitled "Jaundice in pregnancy" was published as a supplement to this journal in 1955. On the basis of the histories and the clinical investigation, including a thymol turbidity test (supposed to be positive only in cases of hepatitis), the 72 cases were diagnosed as viral hepatitis, "jaundice in early pregnancy", "jaundice in late pregnancy" or uncertain. A follow-up study of 61 of the Thorling patients was performed in 1970. The women were characterized with regard to recurrence, type and course of symptoms during all their pregnancies. It was found that the original groups represented heterogenous conditions. Sixteen women fulfilled the criteria of recurrent cholestasis of pregnancy. They experienced pruritus with or without jaundice during all their pregnancies. There was no evidence of exposure to viral hepatitis at the time of the pregnancies. A most interesting finding in the present study was that almost 50% of these 16 women had close relatives who experienced pruritus, often associated with jaundice during pregnancy. Available information from the histories and hospital records did not indicate that drug consumption, biliary tract disease and preeclampsia in general precipitated jaundice or pruritus in any group of women. Bacterial infections may have precipitated jaundice or pruritus in a few cases.

Intrahepatic cholestasis of pregnancy is a condition due to the pregnancy itself. It has been described under several synonyms (10, 13, 21, 22, 23). The symptoms—pruritus and often a slight jaundice—usually appear during the last trimester and may reappear in subsequent pregnancies and during treatment with contraceptive drugs (2, 15). In view of the widespread use of such drugs, the latter observation has stimulated interest in the pathogenesis and long-term prognosis of cholestasis of pregnancy.

In 1955 Thorling (23) described a series of women with symptoms compatible with cholestasis of pregnancy under the synonym "jaundice in late pregnancy". The report also included women with viral hepatitis during pregnancy, jaundice in early pregnancy and pregnant women with non-classified jaundice.

The late L. Thorling, M.D. (d. 1963) wrote his monograph while holding appointments at the Department of Medicine of the University Hospital, Uppsala, and later at the Department of Medicine of Falun Hospital. His cases were collected from the Department of Medicine and the Department of Obstetrics and Gynaecology of the University Hospital, Uppsala, from the Uppsala Hospital for Infectious Diseases and from the Stockholm Hospital for Infectious Diseases. The registration numbers of the hospital records used in Thorling's study are given in the monograph. It was then possible to trace the women through population registers.

The present reinvestigation of the Thorling patients was performed in 1970. As it is unlikely that these women will be pregnant again, this was a unique opportunity to characterize all pregnancies in a group of women who for various reasons had been jaundiced during at least one pregnancy. On the basis of their histories we also tried to investigate the possible existence of predisposing and precipitating factors to itching and jaundice during pregnancy.

The results of the subsequent follow-up study will be reported in forthcoming papers.

MATERIAL AND METHODS

Subjects

Thorling reported on 72 pregnancies in 71 women from 3 hospitals in Sweden (Table I). The clinical diagnoses in

Table I. *Number of women participating in Thorling's and the present study*

	Diagnosis[a]				
	Hepatitis	Jaundice in early pregnancy	Jaundice in late pregnancy	Uncertain	Total
Case no.	1, 3–24	28–33	35–72	2, 25–27, 34	
Thorling's study	23	6	38	4	71
Present study	19	4	34	4	61
Dead	1	1	4	—	6
Not traced	2	—	—	—	2
Unwilling to participate	1	1	—	—	2

[a] According to Thorling.

23 cases with a positive thymol turbidity test (>5 units) was viral hepatitis. The women with negative thymol turbidity tests were separated into two groups. The first consisted of 6 women with severe vomiting and jaundice during the first trimester (jaundice in early pregnancy). The women in the second group were jaundiced during the later months of their pregnancies (jaundice in late pregnancy). Four pregnancies were not referred to any group. One of these, case 27, was the second pregnancy of a woman whose first pregnancy (no. 2 in the Thorling report) was included in the group with hepatitis. In the present paper she is referred to as case 27.

The present investigation comprised 61 of the original Thorling patients. Ten women were not available for the follow-up study. The reasons are given in Table I.

Methods

The women were interviewed with a standard questionnaire. Information was also obtained from hospital records and the Thorling report.

The following principles were used in the evaluation of data. The statement in a hospital record of the presence of a symptom was always accepted. Negative information in a record was considered less trustworthy and was disregarded if the woman was of a different opinion. The absence of notes concerning a symptom was held to be without informative value. Under such circumstances or when records were missing, information was collected from the Thorling report, which was based on hospital records, written interviews and in some instances on personal acquaintance.

Data concerning pregnancies not included in the Thorling study were obtained to some extent from the women themselves. Although their recollections concerning the exact periods of jaundice and itching were approximate, they agreed fairly well with information in available hospital records.

Table II. *The women grouped with regard to the recurrence of symptoms (no. of women)*

	Diagnosis[a]				
Group	Hepatitis	Jaundice in early pregnancy	Jaundice in late pregnancy	Uncertain	Total
1 A	1	4		1	6
1 B			15	1	16
1 C	1		3		4
2 A			9	1	10
2 B	14			1	15
3	3		7		10
Total	19	4	34	4	61

[a] According to Thorling.

RESULTS

Recurrence of jaundice and/or pruritus during pregnancies

The incidence of jaundice and/or pruritus during full-term pregnancies was used to divide the women into three groups. Group 1 consisted of 26 women with such symptoms during more than one pregnancy. The symptoms were most pronounced during the first trimester in 6 (group 1A) and during the last trimester in 20 women. 16 of the latter (group 1B) had symptoms during all and 4 (group 1C) during some of their full-term pregnancies. Group 2 comprised 25 women with symptoms during one out of several pregnancies, 10 of them had a normal (group 2A) thymol turbidity test during the pregnancy studied by Thorling and 15 an abnormal test (group 2B). Of the remaining 10 women (group 3), 9 bore one child each and one had a single pregnancy that ended in abortion.

Table III. *Age distribution (y) at the time of the 1st pregnancy (I), at the pregnancy studied by Thorling (II) and at the present study (III) (mean and range)*

Group		I	II	III
1 A	6	23.5 (18–34)	26.0 (18–34)	44.7 (37–50)
1 B	16	26.8 (21–36)	29.7 (24–37)	47.8 (40–57)
1 C	4	24.5 (20–31)	25.8 (20–31)	43.0 (35–49)
2 A	10	24.8 (19–35)	30.5 (20–31)	48.5 (39–61)
2 B	15	24.8 (19–33)	28.3 (20–33)	49.1 (42–55)
3	10	28.9 (25–34)	29.7 (25–39)	47.7 (43–58)

All patients in Thorling's group "jaundice in early pregnancy" fulfilled the criteria of group 1A (Table II). Out of 27 multiparous women with the diagnosis "jaundice in late pregnancy" in the Thorling study, 15 had jaundice or pruritus during all (group 1B), 3 during some (group 1C) and 9 during none (group 2A) of their other pregnancies.

Fourteen out of 16 multiparous women, supposed to have had viral hepatitis during one pregnancy, had neither jaundice nor itching during their other pregnancies. Such symptoms were encountered in the 2 remaining women, referred to groups 1A and 1C, respectively. The 4 women whose diagnoses were considered uncertain by Thorling reacted in different ways during their other pregnancies (Table II).

Age of the women

The mean age of the women at the time of their first pregnancy ranged between 24 and 27 years in groups 1 and 2 and was 29 years in group 3. The present follow-up study was performed about 20 years after the first pregnancy (Table III).

Number of pregnancies

Altogether there were 171 pregnancies, 27 of which ended in abortions (Table IV). Jaundice and/or pruritus were encountered in 89 of the full-term and in 8 of the abortive pregnancies. Of the latter, those associated with jaundice or pruritus appear to have lasted longer (median value 21 weeks) than those not complicated by such symptoms (median value 12 weeks).

Course of full-term pregnancies

Group 1A. In the Thorling report cases 29, 30, 32 and 33 were included in the group "jaundice in early pregnancy". The diagnosis in case 3 as well as in case 27 during her first pregnancy was viral hepatitis, whereas the jaundice during the latter's second pregnancy was considered to be of uncertain etiology.

Jaundice and/or pruritus occurred only during the first trimester in cases 3, 27 and 32 (Fig. 1, Tables V and VI). The other 3 women had their most severe symptoms in the first trimester but itching usually appeared towards the end of pregnancy.

Groups 1B and 1C. Itching was experienced in all full-term pregnancies in group 1B and in all but one of the afflicted pregnancies in group 1C

Table IV. *Pregnancies complicated by jaundice and/or pruritus*

Week = time of abortion in gestation, median value (range within parentheses)

Group		Full-term (n)		Abortion				Total	
				Compl.		Non-compl.			
		Compl.	Non-compl.	(n)	(week)	(n)	(week)	Compl.	Non-compl.
1 A	6	15	0	1	18	1	10	16	1
1 B	16	34	0	3	23 (8–26)	6	15 (12–16)	37	6
1 C	4	9	5	0	—	1	8	9	6
2 A	10	9	4	1	14	6	11 (8–14)	10	30
2 B	15	13	26	2	21 (20–21)	1	18	15	27
3	10	9	0	1	23	4	12 (6–12)	10	4
Total		89	35	8	21 (8–26)	19	12 (6–12)	97	74

Table V. *Number of full-term pregnancies complicated with jaundice and pruritus*

Group		Total no. of pregnancies	Either symptom	Both symptoms	Pruritus only	Jaundice only
1 A	4	15	15	7	6	2
1 B	16	34	34	19	15	0
1 C	4	14	9	5	3	1
2 A	10	33	9	6	0	3
2 B	15	39	13	11	0	2
3	10	9	9	8	0	1
Total		144	89	56	24	9

(Table V). Its recorded duration was longer in the presence than in the absence of jaundice (medians of 11 and 4 weeks, respectively). Jaundice was never observed before the onset of pruritus. In general the time of onset and the duration of symptoms varied more between women than between the pregnancies of one and the same woman.

The symptoms did not change consistently during subsequent pregnancies. Pruritus might be the only symptom during the first pregnancy whereas a subsequent one was complicated by both jaundice and pruritus or vice versa.

Groups 2A and 2B. Jaundice was observed during 22 full-term pregnancies, 17 of which were complicated by itching. In group 2A itching started before jaundice (median 3 weeks) in all

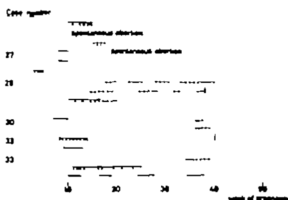


Fig. 1. Periods of jaundice (—) and pruritus ( ) in the women in group 1A. The end of the pregnancy is shown by vertical line.

cases except one. The women of group 2B experienced itching shortly before or at the same time as jaundice (Table VI). In both groups the symptoms started earlier in gestation than in groups 1B and 1C. Three women of group 2A were free from symptoms 7, 8 and 20 weeks before the end of pregnancy. In 10 women of group 2B the symptoms disappeared 1–27 weeks (median 19 weeks) before delivery, but in all the other cases in groups 2A and 2B this did not happen until delivery or soon after.

Group 3. In the cases originally reported as viral hepatitis the symptoms vanished 1, 2 and 16 weeks before delivery. The other women reacted in a way similar to those in groups 1B and 1C (Table VI).

Table VI. *Onset, duration and end of jaundice and/or pruritus in pregnancies with such complications (median value and range)*

Group	Onset of symptoms (week no.)	Duration of symptoms (weeks)	End of symptoms
1 A	See Fig. 1	See Fig. 1	See Fig. 1
1 B	30.0 (4–40)	9.5 (1–40)	At or after delivery
1 C	33.5 (26–38)	8.0 (3–18)	At or after delivery
2 A	28.0 (12–36)	7.0 (3–28)	Before, at or after delivery
2 B	18.0 (6–38)	5.0 (3–34)	Before, at or after delivery
3	31.0 (18–36)	5.0 (2–17)	Before, at or after delivery

Hereditary disposition

Altogether 11 women (18%) reported on relatives with jaundice and/or pruritus during pregnancy. The highest incidence (44%) was encountered in group 1B (Table VII).

Exposure to viral hepatitis

With regard to the risk of contracting viral hepatitis, Thorling distinguished six types of contact. We have used his schedule for classification here with some minor rearrangements in the light of new information. As demonstrated in Fig. 2, the greatest risks (familial contact or direct contact outside the home) were most frequently encountered in group 2B (8 of 15 women). Thorling found no indication that any of the women in

group 1B had been exposed to infectious hepatitis during the pregnancies he studied. Neither did the histories give any evidence that viral hepatitis had complicated the pregnancies not included in Thorling's investigation.

Incidence of cholelithiasis and cholecystitis at the time of jaundice

One woman was cholecystectomized before becoming pregnant, and 4 women in groups 1B and 1C in the interval between pregnancies complicated by jaundice and/or pruritus. Eight out of 17 women investigated with oral cholecystography were found to have cholelithiasis or cholecystitis soon after a pregnancy complicated by jaundice. There is no concentration of positive findings in any group. In only one case (no. 70 group 2A) the jaundice was considered to be due to gallstones.

Incidence of proteinuria and hypertension

Thirty women had hypertension and/or proteinuria during one or several pregnancies. Hypertension only (diastolic BP ≥ 95 mmHg) was recorded just before delivery in 7 women during 7 pregnancies. The elevation was slight to moderate in all instances, the highest diastolic value being 110 mmHg in one case. Proteinuria in the absence of hypertension was found in 16 women during 20 pregnancies. Both symptoms were encountered in 7 women during 11 pregnancies.

The incidence of hypertension and proteinuria was about the same in all groups except group 3 in which 7 out of 10 women had such symptoms. Six of them had hypertension. There was no correlation between the presence of hypertonia and/or proteinuria and the presence or severity of jaundice and pruritus.

Table VII. *Jaundice and/or pruritus during pregnancy in relatives to the women investigated*

Group	Case no.	
1 A	29	One of 2 daughters had severe pruritus during the main part of all her 3 pregnancies
1 B	26	Twin sister had pruritus during 2 of her 4 pregnancies and jaundice and pruritus during 1 pregnancy
	37	Maternal grandmother, mother and maternal aunt had pruritus during several pregnancies
	45	Sister had moderate repeated pruritus during some of 9 pregnancies
	48	One of 2 daughters had pruritus during both pregnancies
	50	Sister had severe pruritus during all 3 pregnancies
	54	Mother had pruritus during 2 of 3 pregnancies
	56	Mother and sister had jaundice and pruritus during all their pregnancies
2 A	65	Daughter of maternal uncle had jaundice and pruritus during 1 pregnancy
	70	Mother had jaundice, pruritus and abdominal pain during her 3rd pregnancy. The symptoms were considered to be due to gallstones. No symptoms after cholecystectomy
2 B	15	Mother had jaundice during 1 pregnancy

In the Thorling report.

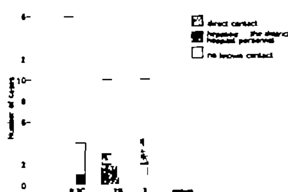


Fig. Exposure to infectious hepatitis during the pregnancies studied by Thorling.

Extrahepatic infections

Symptoms of pyelitis started after the disappearance of jaundice in one woman of group 2B. One case (group 1B) had an infected cystadenoma of the ovary and 4 other women had symptoms of pyelitis (2 from group 1B and 2 from group 2A). In these 5 women the infection and jaundice or pruritus apparently started at the same time.

Hyperemesis gravidarum

All women in group 1A seem to have had hyperemesis during the first trimester of all their pregnancies, with the possible exception of case 27 whose vomiting is described as moderate. Only one woman in group 1B had typical hyperemesis gravidarum. Another woman in this group had almost constant vomiting during her only pregnancy complicated by jaundice.

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THE DUODENAL MICROFLORA IN RELATION TO VARIOUS SYMPTOMS AND MANIFESTATIONS IN PATIENTS WITH EXTRAHEPATIC BILIARY DISEASE

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Abstract. The study comprised 93 patients who had or had previously had some form of extrahepatic disease. Duodenal aspirates from 23 of the patients (class 1) contained more than 10^{3} of aerobic colonic microorganisms per ml. No growth or less than 10^{3} of such organisms and less than 10[illegible] of common pharyngeal species per ml of the aspirate ("normal" microflora, class ?) was encountered in 48 cases. A pharyngeal flora defined as more than 10[illegible] of common pharyngeal species per ml of the aspirate was found in 22 patients (class 3). The incidence of present or previous abnormalities of the common bile duct (choledocholithiasis, stenosis and dilation) was 56% in class 1, and 13 and ?3% in classes 2 and 3, respectively The mean age in classes 1, 2 and 3 was 68.2, 48.3 and 59 1 years, respectively On the basis of previous study it was anticipated that most of the patients in class 1, considerable part of those in class 3 but almost none of those in class 2 harboured microorganisms in the liver and/or the biliary tract. As reflected by laboratory tests and the histology of the liver biopsy specimens, the incidence of liver disease appeared to be unrelated to the type of microflora in the upper small intestine. The incidence of an ESR above 30 mm/hour, fever episodes unrelated to common cold, urinary tract infection and concomitant diseases appeared to be higher in class 1 than in class 2. The significance of these findings with regard to possible relationship between the duodenal microflora and the existence of an infectious process is discussed.

The proximal part of the small intestine of young fasting subjects often contains a small number of staphylococci, streptococci, lactobacilli and fungi (6) The finding of less than 10^{3} of such organisms per ml of duodenal aspirates from patients with extrahepatic biliary disease (cholelithiasis, cholecystitis and choledocholithiasis) almost excluded bacterial infection of the liver and the biliary tract (5) When the duodenal aspirates contained more than 10 of these species per ml, approximately 50% of the patients showed positive cultures from any of the operative specimens from the liver the wall and the content of the gall bladder and the common bile duct The presence of more than 10^{3} of enterococci and members of the family Enterobacteriaceae per ml of the aspirate was, in more than 90% of the patients, compatible with the growth of such bacteria from the specimens obtained at the subsequent operation (5).

The consequences of harbouring bacteria in the liver and the biliary tract with regard to the development of liver disease ha e been discussed by several authors Hobson and Rice-Oxley (8) distinguished a clinical disease, cholangiohepatitis, which was supposed to be caused by bacteria. Although usually secondary to and associated with obstruction of the extrahepatic pathways, the infection was occasionally demonstrated in the absence of such changes. The diagnosis could usually be achieved by biochemical and haematological investigations, but the isolation of organisms from the bile either at the time of the operation or by duodenal aspiration was considered as indubitable evidence for the disease. Cameron and Hou (3), on the other hand, suggested that the li er normally excretes microorganisms into the bile, a phenomenon that may occur in the absence of liver destruction. After a prolonged obstruction the patients usually become "mildly infected" Edlund et al. (4) studied the li er histology in patients operated upon because of cholelithiasis and found the incidence of abnormal findings to be unrelated to whether or not the bile contained bacteria.

The present paper reports on the bacterial microflora in the duodenal aspirates from patients

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The anatomy of the common duct

The anatomical state of the common duct was examined by i.v. or operative cholangiography or, in some cases, exclusively by the surgeon during the subsequent operation. On i.v. cholangiography the width of the common bile duct was measured after correction for magnification. The measurements were made just below the "knee" of the duct, as described by Bodvall (1).

Statistics

The significance of differences between groups was evaluated using the χ^2-test.

RESULTS

A colonic (class 1) flora was found in duodenal aspirates of 23 patients, whereas 48 patients were referred to class 2 ("normal" flora) and 22 to class 3 (pharyngeal flora). The bacterial concentration in duodenal aspirates of patients in classes 1 and 3 ranged widely, the median values being 1.5×10^6 and 1.0×10^4 organisms per ml, respectively. Cultures of aspirates from 22 patients in class 2 showed no growth.

E. coli was the prevailing organism in class 1 (Table II), and in 11 out of 15 patients it was found in a mixture with enterococci or other members of the family Enterobacteriaceae. More than 10^3 pharyngeal organisms per ml were found in the aspirates from 11 patients in class 1. This class also included 5 patients whose aspirates contained anaerobic bacteria. In general the pharyngeal organisms were present as a mixture of several species.

The mean age of the patients in class 1 (65 years) was approximately 10 years higher than in class 3 and 20 years higher than in class 2 (Table I). A class 1 and a class 3 microflora were almost

Table II. *Class 1 bacteria and anaerobic organisms in duodenal aspirates*

Organisms	Total isolations (no. of pats.)
E. coli	13
Aerobacter aerogenes	9
Coliform organisms*	7
Proteus species	3
Anaerobic streptococci	3
Clostridium species	2
E. freundi	1
Enterococci	1

* Other than E. coli.

Table III. *Subclassification of the patients with regard to their age*

Class	<50 y	50–70 y	>70 y
1	—	15	8
2	20	25	3
3	3	16	2
Total	23	56	13

absent in patients below 50 years of age (Table III).

The anatomical state of the extrahepatic biliary pathways

Of the patients referred to class 1, 35% showed choledocholithiasis, stenosis or dilation of the common bile duct. Such changes were found in 8% in class 2, and in 9% in class 3. Including the patients who previously had been operated upon because of choledocholithiasis, 13 out of 23 patients (56%) in class 1 had or had previously had some form of aberration of the common bile duct. Similar findings were encountered in 13 and 23% of the patients referred to classes 2 and 3 respectively.

Excluding the patients with choledocholithiasis, stenosis or dilation of the common bile duct, the width of the choledochus was measured when the i.v. cholangiograms were of suitable density. The value recorded for 13 patients in class 1 was 8.1 ± 3 mm (mean ± S.D.). The corresponding figures obtained for 36 and 16 patients in classes 2 and 3 were 5.4 ± 5 and 6.9 ± 2.5 mm, respectively.

Symptoms

Almost half of the patients in all classes had suffered from abdominal pains during the month preceding the present study. Jaundice had been observed in 4 patients in class 1, in 1 in class 2 and in 2 in class 3. Of these 7 patients 5 had some form of aberration of the common duct. General pruritus was encountered in only a few cases.

Fever periods during the year prior to this study had been experienced in approximately 50% of the patients. The corresponding figure recorded for the preceding month was about 15% (Table IV). The incidence was higher in classes

Table IV *Incidence of fever episodes (F.e.) and morning temperature on the day of the duodenal intubation*

	Class		
	1	2[a]	3[a]
All ages			
F.e. past year	15/23	16/46	14/22
F.e. past month	9/23	6/47	9/22
Morning temp. >36.7 C	11/23	9/46	8/21
50–74 years			
F.e. past year	11/17	13/28	13/19
F.e. past month	8/17	5/28	8/19
Morning temp 36.7°C	8/17	5/28	19

No. of patients with positive findings/no. of patients stud.

1 and 3 than in class _ ($p<0.05$). Among patients 50–74 years of age, fever episodes during the preceding month had occurred more frequently in class 1 than in class _ ($p<0.05$). Almost 50 % of the patients in class 1 had an oral morning temperature above 36.7 C. The temperature was below 37.0 C in the majority of the patients.

Laboratory findings

WBCs exceeding 7 000 cells/mm³ were encountered in 20 % of the patients and this finding was almost equally frequent in all 3 classes of patients (Table V). The mean ESR values recorded for the patients in classes 1, 2 and 3 (means ± S.D.) were 44 ± 38, 18 ± 12 and 4 ± 27 mm/h, respectively. An ESR above 30 mm/h was found in 13 of 22 patients in class 1 and in 6 of 46 in class 2 ($p<0.001$). Among patients 50–74 years of age the corresponding figures for classes 1 and _ were 8/16 and 5/27 ($p<0.05$). With regard to the ESR there was no difference between the patients in class _ and class 3 irrespective of age.

Bilirubin in serum was higher than 1.5 mg/100 ml in 5 patients, 4 of whom belonged to class 1 (Table V). SGOT and SGPT were elevated in 10 and 1_ patients, respectively, of whom 5 were referred to class 1. In all cases the concentrations of the enzymes were lower than 55 U/ml. Increased levels of the alkaline phosphatase in serum were observed in 20 patients, the incidence being approximately the same in all classes (Table V).

The γ-globulins in serum were elevated (>1.7 g/100 ml) in approximately 20 % of the 16 and 19 patients in classes 1 and 2 and in 8 of 14 patients (57 %) in class 3. An abnormal bromsulphalein retention (>5 % at 45 min) was encountered in 8 of 14 patients of class 1, in 6 of 14 in class _ and in 10 of 12 in class 3.

Urinary tract infection

The incidence of urinary tract infection among the 3 classes of patients was estimated on the basis of 1) the frequency of patients reporting histories of such an infection during the year preceding the study and 2) the frequency of patients with positive urine cultures during the study. The history was recorded as positive when the infection had been diagnosed by a physician on the basis of bacterial cultures and/or microscopy of a urine specimen. The number of urine specimens cultured per individual studied during this investigation averaged 1.8 in class 1 and 1.6 in classes _ and 3. These figures were derived by including all negative and excluding all positive cultures, with the exception of the first, from each patient.

Although not significant on a statistical basis the number of patients who reported histories of urinary tract infection tended to be higher in class 1 than in classes 2 and 3 (Table VI). The incidences of positive cultures in classes 1, 2 and 3 was 53, 15 and 18 %, respectively. Among patients 50–74 years of age the corresponding figures for the same classes were 53, 12 and _1 %, respectively. In general the urine cultures yielded growth of a single species of the family Enterobacteriaceae. In 8 patients in class 1 the same

Table V *Laboratory data*

	Bacterial class		
	1	2[a]	3[a]
WBC 7 000/mm³	5/22	7/38	7/20
ESR 30 mm/h	13/22	6/46	7/22
Bilirubin >1.5 mg/100 ml	4/23	0/48	1/21
SGPT >40 U/ml	5/23	4/45	3/22
Alkaline phosph. 3.0 U/ml[b]	8/23	8/47	4/20

No. of patients with positive findings/no. of patients stud.
Bessey–Lowry units.

species of organisms were isolated in the specimens of urine and duodenal aspirate. Among the patients with positive urine cultures 3 in class 1 showed pyelonephritis, 2 diabetes mellitus and 1 hyperplasia of the prostatic gland. One patient in class 3 had chronic inflammation of the urinary bladder and one diabetes mellitus.

Histology of the liver

Liver biopsy specimens were obtained from more than 43 of the patients. Cholangitis defined as an abnormal accumulation of mono- and polynuclear cells in the portal tract was observed in less than half of the patients. The incidence of such changes was almost the same in all classes (Table VII).

DISCUSSION

The present patients, all of whom had or had previously had some form of extrahepatic biliary tract disease, were separated into 3 classes on the basis of the bacterial findings in their duodenal aspirates. This procedure also turned out to be a fractionation with regard to age. The patients with a "normal" microflora (class 2) were the youngest ones, their mean age being 10 years lower than those who harboured more than 10^8 of common pharyngeal organisms per ml (class 3). The latter patients were on the average 10 years younger than those (class 1) who had more than 10^3 of aerobic colonic organisms per ml of aspirate. The three classes of patients also differed with regard to the incidence of choledocholithiasis and anatomical aberrations of the common bile duct. Such changes had been encountered in more than 50% of the class 1 patients and in less than 23% of the patients referred to classes and 3. In the absence of these findings the small differences in the width of the common bile duct that were observed between the three classes of patients may have been due to the differences in age (1).

Recent studies have demonstrated that the microflora in the upper small intestine is relatively constant over long periods (Engstrom, unpublished observations). Bacteria, such as E. coli, that were isolated from the bile at the time of cholecystectomy were recovered in duodenal aspirates several years after the operation (12). On the basis of such observations and of those from the previous study (5), it was anticipated that most of the present patients in class 1, a considerable part of those in class 3 but almost none in class 2 should harbour significant quantities of bacteria in their liver and/or biliary tract.

As reflected by GOT, GPT, bilirubin and the alkaline phosphatase in serum, as well as by histology of liver biopsy specimens, the incidence of liver disease appeared to be unrelated to the type of microflora in the upper small intestine. The percentage of cases with an elevated BSP retention was relatively high among all the patients studied, and on the basis of the laboratory and the microbiological findings it was not possible to distinguish a bacterial cholangiohepatitis as suggested by Hobson and Rice-Oxley (8).

The WBC, the ESR, the morning temperature on the day of the duodenal intubation and the incidence of fever episodes were used as rough parameters in the evaluation of a possible association between the type of duodenal microflora and the existence of an infectious process. The interpretations of the results became in part invalidated when it appeared that the patients in the three classes differed both with regard to their age and to the incidence of concomitant diseases. Using an ESR of 30 mm h as the

Table VI. *Incidence of urinary tract infection*

	Incidence during the preceding year according to the hist		Incidence of positive cultures at the time of the study	
Bacterial class	All pats	Pats 50–74 y	All pats.	Pats 50–74 y
1	6/23	4/17	11/21	8/15
2	4/46	2/28	4/27	4/17
3	3/22	3/19	3/17	3/14

No. of pats. with positive findings/no. of pats. studied.

Table VII. *Histology of the liver. Incidence of cholangitis*

Bacterial class	Needle biopsy specimen[a]	Operation biopsy specimen[a]	Total[a]
1	5/14	3/5	8/19
2	6/14	9/22	15/36
3	5/12	2/3	7/15

[a] No. of pats. with positive findings/no. of pats. studied.

upper normal limit (2), the incidence of abnormal findings was highest in the patient with a colonic microflora irrespective of whether the comparison included all patients or only those 50–74 years of age. The incidence of fever episodes appeared to be higher in classes 1 and 3 than in class 2 but this tendency in class 3 disappeared when the youngest and the very oldest patients were excluded. Within class 1 there was a somewhat higher incidence of fever episodes among the patients with choledocholithiasis or some form of aberration of the common bile duct, and possibly among those with concomitant diseases.

In considering the present findings it is of interest that duodenal aspirates from patients with cirrhosis of the liver often contain an abnormal concentration of colonic organisms (10). The liver normally acts as a bacterial filter presumably by means of a reticuloendothelial system a function that may be impaired in cirrhosis (11). The bacteraemia that develops in cirrhotic patients often shows non-typical features with just a slight elevation of the body temperature (9) Thus, although there may be an association between bacteriaemia and severe liver damage, it was difficult to correlate signs of general infection with an abnormal microflora in duodenal bile of patients with a normal or slightly impaired liver function The incidence of positive urine cultures in class 1 was higher than that earlier reported from this hospital for hospitalized patients of corresponding age (7). Although some of the patients had concomitant diseases that may be associated with bacteriuria, it is an interesting possibility that an abnormal proliferation of bacteria in the bile and the urine in some cases may have reflected an impaired function of the reticuloendothelial system of the liver

ACKNOWLEDGEMENT

The present investigation was supported by Carl-Bertel Nathhorsts vetenskapliga och allmännyttiga stiftelser

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Acta med. scand. Vol. 193 pp. 273–275 1973

THE DUODENAL MICROFLORA AND THE INCIDENCE OF MALABSORPTION IN NON-ICTERIC PATIENTS WITH EXTRAHEPATIC BILIARY DISEASE

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Abstract. The study comprised 93 patients who were cholecystectomized and/or showed some form of extrahepatic biliary disease. An abnormal proliferation of aerobic colonic or pharyngeal microorganisms in the upper small intestine was encountered in 23 and 22 patients, respectively. Minor amounts of unconjugated bile acids were detected in duodenal bile of 3 patients with an aerobic colonic microflora. On the basis of the incidence of malnutrition, diarrhoea, steatorrhoea and abnormal indicanuria it was concluded that the proliferation of bacteria in the upper small intestine had minor effects on the absorption of fat and proteins.

Malabsorption in association with proliferation of bacteria within the lumen of the upper small intestine has been observed in a variety of conditions. It may develop in patients with surgical blind loops, strictures, enteroanastomies, fistulas and diverticula involving the duodenum and the jejunum (4). Malabsorption simulating the blind loop syndrome has also been described in icteric patients with partial biliary obstruction and cholangitis (14). An abnormal microflora in the upper small intestine is commonly encountered in non-icteric patients with extrahepatic biliary diseases (5, 6). Whether malabsorption may develop under such conditions was the subject of the present study.

MATERIAL AND METHODS

The patients were those reported on in a previous study (5). Bacterial cultures from duodenal aspirates were performed in all cases as described recently (6).

With regard to the microflora of the duodenal aspirates the patients were separated into 3 classes. Class 1 comprised 23 patients whose aspirates contained more than 10[illegible] per ml of enterococci and members of the family Enterobacteriaceae, i.e., aerobic organisms usually recovered in the large intestine of healthy subjects. Aspirates from 48 patients referred to class 2 showed no growth or yielded less than 10[illegible] of aerobic colonic organisms and less than 10[illegible] per ml of organisms commonly found in the upper respiratory tract of healthy subjects. Such organisms, in concentrations exceeding 10[illegible] per ml, were recovered in the aspirates of the 22 patients referred to class 3.

The patients were hospitalized and were given the hospital diet without restrictions. During one week some of the patients received a standardized diet of regular type. The intake of tryptophan ranged between 600 and 800 mg per day but was kept constant for each subject. Urine and faeces were collected from the 2nd to the 6th day and analysed for indican and fat. Indican was determined on 24-hour portions of urine according to the method of Bryan (1). Faeces from each patient were pooled and analysed as described by van de Kamer et al. (10). The bile acids in duodenal aspirates were analysed with thin layer chromatography. Aliquots of the aspirates were extracted with chloroform methanol (1 : 1, v/v) for 30 min at reflux temperature. After filtration and evaporation of the solvent under vacuum aliquots of the organic extracts were redissolved in ethanol and spotted on the plates covered with a layer of silica gel. The solvent system described by Gregg (9) was used. The bile acids were detected by spraying the plates with 10[illegible] phosphomolybdic acid and identified by reference to the mobility of standard substances, supplied by Dr J. Sjövall.

RESULTS

The incidence of malnutrition was evaluated by comparing the actual and the standard weights of the patients. The standard weight, calculated as body length in cm minus 100, differed from the actual weight by less than 10% in most cases. It exceeded the actual weight by more than 10% in one patient in class 1 and in two patients in class 2. Underweight was observed in only one patient referred to class 1.

Diarrhoea, defined as a minimum of two loose or bulky stools per day during the week preceding

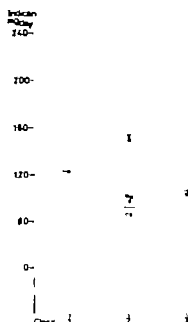


Fig 1 Urinary excretion of indican. Horizontal lines indicate median values.

the present study was reported by one patient each in classes 1 and 3, and in 5 patients in class 2.

The urinary excretion of indican was measured in 9 patients in class 1 in 12 in class 2 and in 7 in class 3. The excretion during 24 hours ranged within wide limits in all classes (Fig. 1) and averaged 123 mg in class 1 97 mg in class 2 and 129 mg in class 3. Indole-producing organisms (E. coli, E. freundi and/or Proteus species) were recovered in the duodenal aspirates of 6 patients in class 1. In these patients the urinary indican exceeded the median value recorded for the patients with a "normal" duodenal microflora (class 2).

Table I. *Patients with unconjugated bile acids in duodenal aspirates. Results of bacterial cultures*

Subject no.	Organisms isolated	Bacterial concentration (organisms ml)
1	E. coli pharyngeal species	5.2 10^{6}
2	E. coli Aerobacter aerogenes	1 0 10^{5}
3	E. coli Proteus species	5.2 10^{6}

Fat was determined in the faeces of 8, 12 and 7 patients in classes 1 2 and 3 respectively. Steatorrhoea (> 17 mEq fatty acids/24 h) was encountered in 2 patients in class 1 and in 2 in class 2. The mean excretion was 13.5 mEq per day in class 1 10.1 in class 2, and 7.2 in class 3.

Spots with Rf values identical with those of cholic and the dihydroxycholanic acids were detected on thin layer chromatograms of the duodenal aspirates of 3 of the 23 patients in class 1. In all instances the concentrations of these acids appeared to be low and roughly only a few per cent of that of the conjugated compounds. The microorganisms isolated from specimens containing free bile acids are shown in Table I. Deconjugated bile acids were detected in one of the two patients with steatorrhoea. Cultures of this patient's aspirates gave growth of E. coli and Aerobacter aerogenes. No deconjugation of the bile acids was observed in the duodenal aspirates of the 46 patients in class 2 nor of the 22 patients in class 3.

DISCUSSION

It is known that intestinal microorganisms are capable of metabolizing important nutrients (15 17). The upper small intestine of healthy subjects is usually populated by only a small number of microorganisms (7). Since most of the digestible carbohydrates, protein and fat are absorbed in the upper part of the digestive tract, it is conceivable that the intestinal microorganisms ordinarily do not interfere with nutrition of the host in any important way. Steatorrhoea and an increased urinary excretion of the tryptophan metabolites, indican and indole acetic acid, were observed in patients with the blind loop syndrome that is characterized by an excessive proliferation of bacteria within the upper small intestine (2, 8, 13, 18). Since the malabsorption may improve upon treatment with antibiotics (12, 16), it appears that the activity of the bacteria in the small intestine under such conditions is of physiological importance.

Recent attempts to explain the mechanism(s) by which a proliferation of microorganisms results in steatorrhoea have focused attention on the effects of certain bacteria upon the bile acid metabolism. These compounds that are excreted from the liver conjugated with glycine and taurine

may undergo bacterial deconjugation already in the duodenum and the jejunum. The unconjugated bile acids are ineffective in promoting fat absorption and may even be cytotoxic. The presence of substantial amounts of free bile acids in small intestinal aspirates of patients showing the blind loop syndrome have been reported by several authors (3). A variety of bacterial species are capable of deconjugating bile acids (11) and producing indole from tryptophan. E. coli species produce indole but do not deconjugate bile acids. Of other members of the family Enterobacteriaceae, E. freundi, but not Aerobacter aerogenes, are capable of producing indoles.

The present study included non-icteric patients who were cholecystectomized and/or had some form of extrahepatic biliary tract disease. The concentration of bacteria in the duodenal aspirates of patients in classes 1 and 3 was within the same range as reported for icteric patients with partial biliary obstruction who showed a marked steatorrhoea (14). The metabolic consequences of the bacterial proliferation in the upper small intestine of the present patients appeared to be of minor importance. There was no underweight except for one patient in class 1. Steatorrhoea was encountered in only 2, and a slight deconjugation of bile acids in 3 patients in class 1. The urinary excretion of indican showed a tendency to be somewhat higher in class 1 than in the other two classes. From nutritional point of view this extra decomposition of tryptophan should be insignificant.

ACKNOWLEDGEMENT

The present investigation was supported by Carl-Bertel Nathhorsts vetenskapliga och allmännyttiga stiftelser.

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Acta med. scand. Vol. 193 pp. 277–280, 1973

EFFECT OF G-SUIT IN TREATMENT OF POSTURAL HYPOTENSION

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Abstract. Continuous, daily use of a modified aviator's g-suit has effectively prevented severe symptoms of postural hypotension in two cases where other methods of treatment had failed. Hemodynamic effects were studied on a tilt table. Inflation of the g-suit resulted in considerably higher systemic arterial pressure at heart level in the head-up position. Increases were recorded in pulmonary arterial pressure and cardiac output. It is suggested that the g-suit exerted its effect mainly by redistribution of blood volume into the intrathoracic compartment.

In postural hypotension the mean arterial pressure at the level of the heart decreases following a change from the recumbent to the upright body position. Normally an increase of the peripheral vascular resistance compensates for the fall in cardiac output which is due to shift of blood out of the thorax as one assumes the upright body posture (4). The arterial pressure at heart level remains nearly unchanged because of a reflex increase in sympathetic nervous activity producing arteriolar constriction, enhanced cardiac contractility and increased heart rate. In postural hypotension the intrathoracic blood depletion is not balanced by such an increase in sympathetic activity, there is little change in the heart rate and the systolic and diastolic arterial pressures at the level of the heart are reduced so as to interfere with the perfusion of the brain about 30 cm above the heart.

Treatment of postural hypotension may be causal, e.g. if secondary to hypovolemia or use of ganglionic blocking agents. In many cases, however, there is an underlying disease which may be irreversible, as in nervous system degeneration (5, 8) and the treatment of severe postural hypotension in such cases may present great difficulties. It is important to consider factors which may pathologically increase the amount of blood pooling in the dependent parts of the body as one stands up, like varicous leg veins and prolonged bedrest. In the presence of such factors the impaired sympathetic compensatory mechanisms will be even more insufficient in preserving the perfusion of the brain. Surgical treatment of a postphlebitic syndrome, or leg muscle training, which can be performed horizontally may be helpful in these circumstances. However, even in cases of postural hypotension where there is no obvious reason for the dependent blood pool to be greater than normal, elastic bandages, externally compressing the legs have been found to be of some benefit, at least temporarily (6). By providing external support to the leg veins, and thus reducing their compliance, such bandages might serve to increase the intrathoracic, effectively circulating blood volume in the upright posture. For the same purpose the aviator's g-suit, which applies pressure also to the lower part of the abdomen, has been tried in postural hypotension (1). The g-suit was used by Bevegård et al. (2) as a means of studying the physiological characteristics of postural hypotension. The possibility of more permanent use of pressure suits to prevent severe symptoms of postural hypotension has been doubted (1, 6), although Sieker et al. (9) have reported the effective treatment of postural hypotension in four cases by means of tailored elastic pressure garments designed to apply pressure over the body from the waist and down.

The present communication reports the successful use of a standard modified aviator's g-suit in the treatment of two cases of severe postural hypotension where extensive previous treatment had failed.

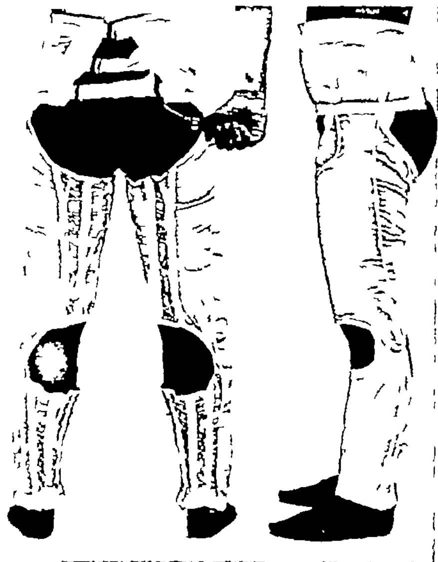


Fig 1 Design of g-suit

DESCRIPTION

The g-suit applies external pressure to the legs and lower part of the abdomen by means of pneumatic, communicating bladders, distributed over the lower part of the body as shown in Fig. 1. The bladders are inflated with a hand bulb prior to assumption of the upright body posture. The suit, which is commercially available (CAMP Scandinavia, Stockholm), has the same design as the aviator g-suit, but its construction has been modified so as to make possible its continuous, daily use with minimal discomfort.

CASE REPORTS

Case 1

Male, aged 69, who in about 1963 first experienced increased tiredness and dizziness. Occasionally he fainted on standing or even sitting. He had to spend most of the day in a lying position. Hypotension had been known for six years. Treatment with N-ethyl-normethaoxidrine and dihydroergotamine was tried without success. Some improvement was obtained from support to the leg veins by an elastic roller. One year ago he received a g-suit which he has been using daily. The g-suit has markedly improved his condition and he has never fainted in the last year. His tiredness is still a problem but is less pronounced. He is now able to go out for a walk on his own.

Case 2

Female, aged 76, with episodes of dizziness since 1956, sometimes falling to the ground. Clinical investigation nine years later revealed marked hypotension on standing, and subsequently she was admitted to hospital several times because of postural hypotension. Treatment with dihydroergotamine, 9 α-fluorhydrocortisone and vasopressin spray met with only limited success. In order to counteract

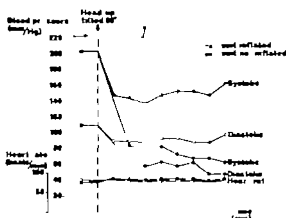


Fig. 2. Heart rate and systemic arterial pressure responses to head-up tilt in case 2 with and without inflation of the g-suit.

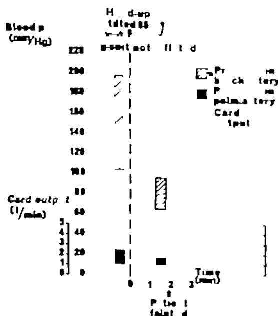
sudden giddiness when occasionally walking with her daughter the patient often had to bend forward as if to fasten her shoestrings. She has been completely relieved from these troubles since she started using g-suit eight months ago, and now she can manage her own shopping.

METHODS

The patients were studied on tilt table with foot support and bindings to prevent the patients from falling case of impending blackout following tilt from the horizontal to the vertical. After recording the heart rate and arterial BP (using standard equipment for direct BP recording) with the patients recumbent, they were tilted up to have the gravitational force vector act in the head to foot direction. Arterial BP and heart rate were recorded continuously. Comparisons were made with and without having inflated the g-suit before tilting the patients. They were studied with the suit inflated at different pressures to obtain the pressure at which hypotensive symptoms over several minutes of observation were clearly improved. Usually such improvement was obtained at pressure that was well tolerable to the patients. Recordings were also made of the pulmonary arterial pressure and cardiac output in case 2 (using Beckman Cardiodensitometer) before and following tilt of the patient with the g-suit inflated to the suitable pressure, and these values, like those for systemic arterial pressure and heart rate in both patients, were compared with the corresponding variables with the g-suit uninflated. The reference point for BP measurements was at the mid-thoracic level and the insertion of the 4th rib at the sternum.

RESULTS AND DISCUSSION

Both patients demonstrated the same hemodynamic characteristics. Following head-up tilt of the patient with the g-suit inflated, there was a moderate fall of the systemic arterial systolic and diastolic pressures at heart level. This fall in pressure was considerably less than that with the g-suit uninflated. This is shown in Fig. 2, which also show that the response of the heart rate was unaffected by the external pressure exerted by the g-suit. These findings, which agree with those of Bevegård et al. (2), express the disturbed



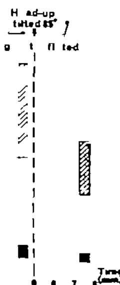


Fig. 3. Systemic and pulmonary arterial pressures and cardiac output in case 2 before and after head-up tilt with g-suit uninflated and inflated.

sympathetic cardiovascular control in postural hypotension, the g-suit probably serving to counteract orthostatic displacement of blood volume out of the thorax, so that an adequate venous return and cardiac stroke output can be maintained. In case 2 (Fig. 3), with the g-suit inflated, there was a fall in cardiac output following head-up tilt of 1 6 l/min, which is within normal limits (3) With the patient in the horizontal position the cardiac output was the same (4.6 l/min) when the g suit was uninflated as when it was inflated. After head-up tilt with the suit still uninflated, syncope occurred within 2 min, and no cardiac output measurement could be performed. The increase of the total peripheral vascular resistance which was afforded by the inflated g-suit in the head-up position was probably due rather to an increase of cardiac output than to external support of the arterioli (9) because of increased tissue pressure.

Fig. 3 illustrates that the pulmonary artery pressure fell slightly upon head-up tilt, both with and without the g-suit inflated. However with the g-suit inflated, the pulmonary artery pressure was higher both in the lying and head-up positions. As earlier reported by Sieker et al. (9) it was observed that the sudden reduction of the pressure in the g-suit in the upright posture resulted in an immediate fall in the pulmonary artery pressure. The most likely explanation seems to be a decrease of the output of the right heart secondary to diminished diastolic blood inflow from the lower parts of the body

Postural hypotension is in many cases a steadily progressive disorder (1). Conventional treatment, with elastic bandages, 9 α-fluorhydrocortisone or dihydroergotamine may be successful in the early stages of the disease, but often fails when it becomes more advanced In the two cases presented in this report, previous treatment could not prevent the development of severe incapacitation, and the application of the g-suit dramatically improved these patients. In our experience these advanced cases of postural hypotension, once given the g-suit, have little to gain by adding vasoconstrictor drugs, e.g. dihydroergotamine or ephedrine. Theoretically measures aimed at increasing the blood plasma volume, like sleep in the sitting position (7), or administration of 9 α-fluorhydrocortisone, should be useful even in the most advanced stages of the disease and might well be combined with the use of a g-suit.

It was suggested by Bannister et al. (1) that the g-suit would be too uncomfortable to wear might cause closure of limb blood vessels and, by reducing the extent of the fall in BP might impair the degree of orthostatic tolerance by various reflex mechanisms. The successful, almost daily use for more than one year of the modified g-suit used in this study did not support the above considerations, and it is suggested therefore that the g-suit is a valuable complement to the therapeutic arsenal in severe postural hypotension where other methods of treatment have failed.

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Acta med. scand. Vol. 193 pp. 281–292, 1973

EFFECTS OF ALPRENOLOL AND ISOSORBIDE DINITRATE IN ANGINA PECTORIS

A Comparative Study with Methodological Considerations

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Abstract. Seventeen patients suffering from exertional angina pectoris according to their records, and confirmed by exercise testing, ha been subjected to 40-week study (including 13-week pretrial period) for assessing the effects of placebo, isosorbide dinitrate (ISD) 5 mg q.i.d. orally and alprenolol (ALP) (an adrenergic β-blocker) 100 mg q.i.d. Variables measured were *nitroglycerin consumption, scale rating of subjective symptoms*, casual exercise testing, and step counter readings of daily activity. These variables were used to calculate "severity index" and "total response index". ISD was found to have some, possibly treatment, beneficial effect, hich was related to decrease in systolic BP. The effect of ALP as significantly better in 14 of 17 patients responding to the drug, both with regard to nitroglycerin consumption, severity index, total response index and exercise testing. Placebo tablets reduced nitroglycerin consumption by about 25%, over 10 eeks, but the severity of angina was not influenced. Side-effects were few on all drugs. Only headache as reported significantly more often during ISD therapy. The methodology of testing treatment efficacy in angina over prolonged periods is discussed, and the severity index is compared with conventional variables such as attack counting, subjective rating and casual exercise testing. The severity index correlates ell with the total response index. It is concluded that the severity index, although theoretically the most suitable variable, is in practice difficult to determine, and that total response index probably is as sensitive, and easier to obtain on the basis of conventional variables.

With the introduction of adrenergic β-receptor blockers in clinical practice, potent drugs for prophylactic use in angina pectoris ha e become available (15). No other antianginal type of drug seems to ha e been subjected to such extensi e and well controlled studies. However thus development has also led to discussion of the methods of testing antianginal therapy and their ability and attempts have been made to improve these methods. It has also become apparent that older prophylactic drugs, generally called coronary vasodilators are often poorly documented in spite of their wide use.

The purpose of the present trial was primarily to evaluate the long-term efficacy of a new adrenergic β-blocker alprenolol (ALP) (H 56/28, Aptin® Hässle, Göteborg, Sweden) (1, 11) in patients with angina pectoris, and to compare its effects with those of a long-acting nitrate compound, isosorbide dinitrate (ISD). For the sake of the design of the trial, an oral dose of 5 mg was chosen to be given four times daily which is slightly *higher* than the dosage level commonly used in Scandina ia, i.e. 5 mg three times daily.

In the process of designing th trial w ex tended the questions to be studied also to include methodological considerations, iz. a) what is the effect of placebo medication, and b) do currently used variables, such as nitroglycerin consumption and attack counting, subjective rating and casual standardized exercise testing, truly reflect an improvement of the patient, defined as decreased severity of angina and or an increase in daily physical activity? In an attempt to evaluate question) we ga the placebo during a run-in period to half of the patients, during which the other half received ISD. To answer question b) we used a *step counter carried by the patients*. This is the first study to our knowledge, which reports the use of such de ice in an angina study.

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Fig 1 Design of the study

MATERIAL AND METHODS

The design of the trial is shown in Fig. 1 About 100 patients, previously hospitalized, ho in their records were reported to have typical history of angina pec toris according to the WHO criteria (31), ere selected from pool of patients attending the Out-patient Clinic of Tampere Central Hospital. After exclusion of patients on digitalis or with intolerance to ISD, or having asthma, emphysema, bronchitis, diabetes or enlarged heart on X ray e. relative olume of more than 500 ml/m² calculated according to Larsson and Kjellberg (20), as ell as those having less than one attack per day 24 patients remained. Patients with previous cardiac infarction or hypertension were not excluded. These patients then attended regular controls for mean period of 3 months, continuing with their usual antianginal therapy as ell as sublingual nitroglycerin Mean nitroglycerin consumption (tablets/week) for this period was calculated from the prescriptions issued by the physician. The patients were *not* informed that they were to take part in a therapeutic trial until this pre-run-in period had finished and the trial proper started th the run-in period (Fig. 1). The pre-run-in period may be considered sufficient to establish optimal rapport between doctor and patient (6).

T wards the end of the pre-run-in period bicycle ergometer test was performed. Five of the patients were *not* restricted by angina pectoris as evidenced by pain and ischemic ECG changes during these tests. They were thus discarded, leaving 19 patients to start the run-in period (Table I). The study thus comprises patients with the *subjecti* symptom of angina pectoris and *objecti* changes of ischemic ECG changes *and* chest pain on exercise.

The patients entered the study between Nov 1968 and July 1969 The last patient completed the study in Jan. 1970. They ere asked to take part in trial f different drugs, each of hich would be given to them for one month and in such way that neither the patient nor the physician knew which drug was actually tried during any period. All patients consented to this procedure. They were told to use nitroglycerin as before. N other drugs but the test drugs were used except by ne patient (no. 14), who had antihypertensi treatment. The duration of the run-in period ranged from 8 to 12 weeks. One patient (no. 17) soon worsened and refused to continue; he turned out to have had ISD therapy The remaining 18 patients completed the run-in period. Their age was 52.0 ± 1.7 years (mean ± S.E.M., range 41–69). The verage duration of angina was 5.0 ± 0.7 years. The nitroglycerin consumption during the pre-run-in period was 12.1 ± 1.6 tablets week (range 5–28). 14 patients had had at least one myocardial infarction.

During the run-in as well as cross-over periods, the patients were seen every four weeks by the physician At control visits, performed between 9 and 10 a.m physical examination of the heart and lungs was done and BP was recorded in the supine and standing position Standardized questions regarding effect (severity of attacks, efficacy of sublingual nitroglycerin and subjecti effort capacity) and side-effects were asked. The patients were asked to mak comparisons with the previous period. An overall judgement was also requested.

Nitroglycerin consumption as noted daily in patient diaries, hich were changed at each control visit. The patients were equipped th step counters at the beginning of the run-in period. These were carried in breastpocket by men and attached to the brassière, between the breasts, by women, and the positions of the hands of the counter were noted on a picture each eek before resetting. One patient (no. 7) could not ear step counter because of his work.

The nitroglycerin consumption, the subjective rating and the step counter values have been used to define two

Table I. *Data for the 19 patients starting the study*

a.p. = angina pectoris, NIT = nitroglycerin, PRI = pre-run-in, RI = run-in

Pat. no.	Age (y)	Sex	Duration of a.p. (y.)	Previous infarction	NIT consumption in PRI period (tablets/week)	Other antianginal therapy in PRI
1	40	♂	4	−	13	—
2	52	♂	8	+	8	Myangin 1 tabl. 3
3	51	♂	2	+	24	—
4	44	♂	2	+	14	—
5	47	♂	5	+	5	—
6	69	♂	5	+	25	ISD[d] 5 mg. 3
7	41	♂	6	+	7	Myangin 1 3
8[a]	55	♂	12	+	10	ISD[d] 5 mg. 3
9	48	♂	5	+	9	—
10	51	♂	4	−	10	ISD[d] 5 mg. 3
11	48	♂	2	+	28	—
12	55	♂	10	−	11	—
13	56	♂	8	−	8	ISD[d] 5 mg. 2
14	49	♂	5	+	8	—
15	57	♂	2	+	7	—
16	53	♂	2	+	8	ALP[d] 50 mg. 3
17	Dropped out after 8 weeks of RI					
18	65	♂	6	+	12	—
19	55	♀	2	+	10	Myangin 1 3

Rash on ALP. Later much worse a.p. when on ISD. Excluded from cross-over period calculations. He later developed rash when given ISD also.
Hypertensive, also took α-methyldopa and diuretic throughout the trial.
Contains glyceryl trinitrate 0.5 mg, glyphylline 0.1 g, papaverine hydrochloride 40 mg, atropine hydrochloride 0.2 mg, phenobarbitone 20 mg.
[d] These patients were prescribed ISD prophylactically but no counting of the consumption was done in the PRI period.
Commercially available preparations.
Of the patients taking placebo during the RI period, one had Myangin, one ISD and one ALP during the PRI period.

Table II. *Nitroglycerin (NIT) consumption during the trial (run-in (RI) and cross-over periods)*

NIT consumption (tablets/week)

Pat. no.	1st week	Week 1–4	Week 5–8	Mean RI	1st ALP	1st ISD	2nd ALP	2nd ISD	Mean ALP	Mean ISD
Placebo during RI										
1	7	10.8	16	14.7	8.5	9.8	7.5	12.3	8	11.1
2	8	7.8	5.3	6.1	2.3	1.0	0.8	1.5	1.6	1.3
4	7	7.5	10	8.3	11.5	9.3	6.5	12.0	9	10.7
7	8	7	6.8	6.8	6.3	5.5	6.0	10.3	6.2	7.9
9	6	5	4.5	4.6	3.0	4.0	4.0	4.5	3.5	4.3
13	6	7.3	5.8	6.5	3.8	4.0	3.5	5.3	3.7	4.7
15	7	8	7.3	7.6	5.3	6.3	3.3	4.3	4.3	5.3
16	2	2	3	3.3	2.0	10.5	9.8	3.5	5.9	7
18	8	5.5	2.5	4.0	5.8	2.3	0	2.3	2.9	2.3
ISD during RI										
3	26	25.3	17.5	21.1	14.3	12.3	11.5	12.3	12.9	12.3
5	4	5	6	5.5	3.0	2.5	1.5	2.3	2.3	2.4
6	16	17.8	16.5	17.7	7.8	17.3	13.3	17.5	10.6	17.4
8	4	2	4.3	3.2	4.5[a]	2.3[a]	—	27.3[a]	—	—
10	8	8	3.8	5.9	3.8	4.3	5.5	8.0	4.7	6.2
11	32	23.5	15	19.9	50.8	27.5	21.8	29.8	36.3	28.7
12	19	9	9.8	9.4	5.5	4.5	11.5	11.5	8.5	8
14	4	4	1	2.7	1.5	3.3	1.5	1.3	1.5	2.3
19	12	9.3	4.3	6.8	1.8	1.0	0.5	9.8	1.2	4.9

[a] Not included in the statistics.

$p < 0.05$

Table VI *Mean % changes of the severity index calculated as the quotient nitroglycerin consumption/step counter readings*

The severity index of the 1st week is taken as 100 % for each individual patient. Patients 7 and 8 not included

Group	First 4 weeks of RI	Last 4 weeks of RI	1st ALP	1st ISD	2nd ALP	2nd ISD	Both ALP	Both ISD
All pats.	—	—	66	104	74	90	72	94
				$p = 0.10$	$p = 0.05$		$p = 0.02$	
Placebo	116	123	86	162	101	111	95	130
				$p = 0.05$			$p = 0.02$	
ISD	90	55	45	49	47	68	46	58
	$p = 0.05$				$p = 0.05$		$p < 0.10$	
Confidence limits for difference placebo — ISD group		$p < 0.05$	$p < 0.05$	$p < 0.02$			$p < 0.05$	$p < 0.05$

to ISD periods, neither were there any significant changes in subjective rating when patients changed from run-in to cross-over periods in either group.

Step counter values and severity index

The average weekly step counter readings are given in Table V The readings ranged from 12 000 to 166 000 during the first week in different patients. There were statistically significant changes between single periods. When both ALP and both ISD periods were considered together the reading during ALP treatment was 8% higher than during ISD treatment. This difference was of borderline significance ($p < 0.10$)

Due to the large individual baseline differences, the changes throughout the trial have been related to the first week of the run-in period when the patients started to use the step counters. (It is assumed that no large step counter changes from the pre-run-in period occurred during this week.) The average nitroglycerin values were divided by the average step counter values. The values so obtained for the first week were taken as unity (100% for each patient) A decrease of this ratio, which we call *severity index*, indicates diminished severity of angina. The values of this index for the other periods have been related to the 1st week value. In Table VI mean values of these severity index percentage changes have been listed for the placebo and ISD groups and for all patients from the cross-over periods onwards Periods following one another have been compared and *p*-values for the changes have been entered in the Table.

In the *placebo group* there was a significantly lower mean severity index in the second ALP period than in the first ISD period. Taking all ALP and ISD periods together the severity index is significantly lower on ALP ($p = 0.02$)

In the *ISD group* the severity index falls significantly from the first four to the second four weeks, then remains at a low level with insignificant changes until the second ISD period, when there is a significant rise from the ALP period. Taking all ALP and ISD periods together the difference is of borderline significance ($p < 0.10$)

For *all patients* the severity index is significantly lower on ALP than on ISD treatment ($p = 0.02$)

During the second four weeks of the run-in period the mean severity index in the placebo group was significantly higher than that of the ISD group ($p < 0.05$) This was also true for both ALP and ISD treatment when all cross-over periods are considered, the severity index being *lower* in the ISD group than in the placebo group ($p < 0.05$).

Heart rate and blood pressure

Resting values. Mean values for HR and systolic and diastolic BP are given in Table VII for the pre run-in and run-in periods (placebo and ISD groups) and in Table VIII for the cross-over periods (ISD and ALP treatment)

During the pre run-in and run-in periods the systolic BP was significantly higher in the ISD group than in the placebo group. Diastolic BP was significantly lower in the placebo group only in the run-in period, supine position. HR tended

to be lower in the placebo group but the difference was not statistically significant.

There were no significant changes from pre-run-in to run-in periods in either group, although the BP tended to decrease with time.

When changing from supine to erect position, HR increased consistently by about 8 beats/min, both in the placebo and the ISD group, in the pre-run-in and run-in periods alike.

In the cross-over periods the mean HR was lower on ALP than on ISD treatment (−6.2% supine, $p < 0.10$, and −9.2% standing, $p < 0.001$). When changing from supine to standing position, HR increased 10 and 7 beats/min on ISD and ALP respectively (difference not significant). This shows that the orthostatic HR reaction is very little influenced by ALP.

BP values were not significantly different: mean readings were 147/91 for both ALP and ISD nor did they change significantly from supine to standing position on either ALP or ISD treatment.

Exercise values. As different final work loads were attained by the patients on different drugs, only HR and BP on comparable work viz. at 4 min on 300 kpm/min, were calculated and compared. ALP caused a mean decrease of 6.7 beats/min from a control value of 104.4 beats/min. ISD and placebo caused no significant

Table VII. *HR and systolic and diastolic BP at rest in the pre-run-in (PRI) and run-in (RI) periods (mean ± S.E.M.)*

	PRI		RI	
	Supine	Standing	Supine	Standing
HR (beats/min)				
Placebo	71.2	79.8	73.2	82.7
	±2.11	±2.20	±1.91	+1.66
ISD	77.6	85.9	79.7	87.5
	±3.15	±3.16	±2.41	±2.13
Systolic BP (mmHg)				
Placebo	148.3	143.3	139.2	136.3
	±5.27	±6.01	±2.40	±3.14
	$p < 0.05$	$p < 0.10$	$p < 0.001$	$p < 0.005$
ISD	168.3	160.0	159.1	153.8
	±7.26	±6.42	±4.83	±4.60
Diastolic BP (mmHg)				
Placebo	93.3	94.4	92.5	92.5
	±4.41	±2.42	±1.93	±1.93
			$p < 0.05$	
ISD	98.9	100.0	98.6	97.5
	±5.12	±2.89	±2.11	±2.08

Table VIII. *HR and systolic and diastolic BP at rest on ALP and ISD treatment in cross-over periods (mean ± S.E.M.)*

	Supine	Standing
HR (beats/min)		
ALP	72.5	79.9
	±2.05	±1.57
	($p < 0.10$)	($p < 0.001$)
ISD	77.3	87.8
	±1.56	±2.46
Systolic BP (mmHg)		
ALP	146.6	148.4
	±3.69	±3.94
ISD	147.1	142.2
	±4.09	±3.69
Diastolic BP (mmHg)		
ALP	90.9	98.2
	±2.13	±2.43
ISD	90.7	100.7
	±2.15	±1.77

change of exercise HR. Systolic BP was on the average lowered significantly by ALP from 181.5 to 153.6 mmHg ($p < 0.001$) and by ISD from 189.2 to 177.8 mmHg ($p < 0.05$). The mean decrease on ALP (27.9 ± 4.77 S.E.M.) is significantly larger than the mean decrease on ISD (11.4 ± 4.78, $p < 0.05$).

Effort tolerances

Table IX gives control values of total work and the product of systolic BP and HR at 4 min on 300 kpm/min. It also gives the percentage deviations from control values on placebo, ALP and ISD treatments.

There was significant increase from control values in total work and decrease of the pressure rate product on ALP ($p < 0.05$) but no significant changes on ISD or placebo of either variable.

A total work comparison between ALP or ISD and placebo could only be made in 5 and 4 patients, respectively the mean diferences from placebo being +58% on ALP and −15% on ISD. It may also be noted that all 5 patients who had double ISD tests performed best in the first test, i.e. in the run-in period.

It has been reported that as much as 20% increase in total work may be within the normal variations of this type of exercise testing in anginal patients (22). Setting ±20% as the limits for positive and negative responses, it is found

Table IX. *Control values of total work (TW) and the product of systolic BP and HR at 4 min on 300 kpm/min, pressure-rate product (PRP), and % changes on different treatments*

Pat. no.	Control values		Percentage changes on					
			Placebo		ALP		ISD	
	TW (kpm)	PRP	TW	PRP	TW	PRP	TW	PRP
1	4 200	1 998	+28.6	+ 4.6			+14.3	+22.7
2	3 900	1 683	−23.1	−27.4	+38.5	−13.5		
3	2 200	2 940					−10.0	− 4.6
4	10 800	1 564	± 0	− 7.9			± 0	−22.7
5	7 200	1 617			+25.0	+ 2.0	± 0	± 0
7	6 300	1 881	−43.9	+13.0			−67.7	−12.3
8	3 000	2 376					± 0	−22.8
9	9 000	1 815	− 5.0	−15.5			−10.0	− 2.1
10	4 800	2 090			+50.0	−21.9	+50.0	−21.9
11	10 800	1 698					+16.7	−10.1
1	7 200	1 824	+66.7	−12.3	+50.0	−36.8		
14	4 000	2 240					− 6.2	+18.1
15	2 800	2 400	−10.7	− 5.7	+50.0	−22.0		
16	7 500	2 100	± 0	−10.0	+20.0	−32.1		
18	2 700	1 800	−53.7	+ 2.7	± 0	−33.3		
19	1 200	3 066					± 0	−13.4
Mean	5 475	2 068	− 4.6	− 6.5	+33.4	−22.5	− 1.2	− 6.3
S.E.M.	754	111	12.11	4.00	7.27	5.10	8.41	4.65
P-values for change from control (Wilcoxon test)			N.S.	N.S.	=0.05	<0.05	N.S.	N.S.

Differences in TW ALP versus ISD significant ($p < 0.01$), ALP versus placebo significant ($p < 0.05$), ISD versus placebo not significant. — Differences in PRP: ALP versus ISD significant ($p < 0.01$), ALP versus placebo significant ($p < 0.01$), ISD versus placebo not significant.

that ALP increases total work in 6 out of 7 patients (84%) ISD in 1 out of 11 and placebo in 1 out of 9. This is statistically significant for ALP versus both ISD and placebo ($p = 0.01$ by Fischer exact probability test).

Total response index

The mean values for nitroglycerin consumption, subjective daily rating and step counter readings for placebo treatment (run-in period) and ALP and ISD treatment (cross-over periods only) as given in Tables II, IV and V have been compared with pre-run-in values or to first run-in week values. Depending on the direction of change from these values, each patient is assigned +1 or −1 for each variable. The sum of these values constitutes the total response index. Table X gives the distribution of patients with different total response index values. Placebo treatment has been included for comparison, although the number of patients is too small to permit χ^2-analysis.

Table X. *Total response index (TRI) distribution*

	TRI		
	+3	+2 or +1	0 or −
ALP	9	4	4
ISD	3	11	3
Placebo	2	3	4

ALP vs. ISD $\chi^2 = 6.41$ $p < 0.05$.

ALP produces a significantly better total response index than does ISD ($\chi^2 = 6.41$, $p < 0.05$). (The result is the same if the non-responders on both drugs are excluded from the calculations.)

The total response index has been compared with the severity index to determine the interrelationships between the two indices. This comparison has been expressed in Fig. 3 in the form of a Venn diagram as suggested by Feinstein (11). For ALP treatment the severity index falls out positively in all total response index positive patients, and picks out yet another one (no. 3). For ISD treatment the severity index fails to detect



Fig. 3. Interrelationship between decrease of severity index and low and high total response index in 16 patients who used the step counter. Circle = decrease of severity index, large square = total response index 3+, small square = total response index 2+ or 1+. The figures denote number of patients.

two low-grade total response index positive patients (nos. 1 and 4). The two non-responders (nos. 13 and 16) are not selected by any of the indices. There is a third non-responder (no. 7) who did not carry a step counter.

From Table IV the individual mean percentage changes of the severity index from the first run-in week were calculated. An arbitrary limit of 40% decrease was chosen to divide the severity index response quantitatively. The interrelationships to total response index have been depicted in Fig. 4 with another Venn diagram, and show that there is good quantitative correlation between the total response index and severity index responses.

Side-effects

The patients were questioned concerning a specific number of symptoms at each control visit, and the occurrence of side-effects reported is given in Table XI. No symptom occurs significantly more often on ALP or ISD compared to placebo, except for headache, which occurs more frequently during ISD treatment ($p < 0.05$). No complications necessitating withdrawal of treatment occurred, except in patient 8, who was withdrawn from the cross-over part of the study (Table I).

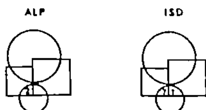


Fig. 4. Interrelationship between large and small decrease in severity index and low and high total response index in 16 patients who used the step counter. Large circle = severity index decrease > 40%, small circle = severity index < 40%. Other symbols as in Fig. 3.

Table XI. *Side-effects during the entire trial calculated as patient reports per 100 treatment periods*

Symptom	ALP (35 periods)	ISD (58 periods)	Placebo (24 periods)
Heart sensations[a]	8.6	13.8	4.2
Dyspnea	11.4	19.0	12.6
Edema	—	—	4.2
Tiredness	5.7	6.9	8.4
Insomnia	2.9	1.7	4.2
Vertigo	5.7	6.9	—
Nausea	5.7	—	4.2
Headache	5.7	17.3	8.4
Dyspepsia	2.9	—	—
Rash	2.9[c]	1.7[c]	—

[a] Feeling of bradycardia, tachycardia, irregularities or palpitations.
[b] Incidence on ISD significantly higher ($p < 0.05$).
[c] Same patient.

Heart size as judged by chest X-rays before and after the entire study did not change (mean before 422 ± 11.5 after 428 ± 11.5 ml/m^2 BSA).

DISCUSSION

Methodology of assessing antianginal drugs

The assessment of long-term continuous antianginal therapy has mainly been based on such variables as subjective rating of the severity of angina, and of attack and/or sublingual nitroglycerin counting. It is true that exercise testing has been quite widely advocated and is probably the best objective acute phase measurement, but this assessment reflects a casual determination of something which is assumed to vary considerably in the angina patient. Thus casual exercise testing may not reflect what is happening during a prolonged period of weeks or months on a treatment under study.

A decrease in attack rate is generally considered to be a prerequisite for assigning antianginal efficacy to a drug. It is quite remarkable that this axiomatic proposition is seldom challenged, as the attack rate must necessarily vary with daily activity. A strong hypnotic drug, which puts the patient to sleep all the time, would logically be

an excellent antianginal drug, as the attack rate would go down to almost zero. Attack counting is therefore of limited value as a quantitative measure if it cannot be related to daily activity. (This obviously does not hold true when angina pectoris caused by psychic stress is considered—this discussion refers to patients with *mainly* exertional angina.)

Subjective rating is also a poor quantitative measure, in spite of rating scales and standardized questioning. One main reason is probably that it usually involves a comparison with pre-drug conditions, and patients may recall these differently under different conditions. As qualitative measures, however, attack counting and subjective rating may serve adequately.

The purpose of using the step counter was to see whether this could be used to quantitate the daily physical activity and thus find a quantitatively suitable measurement for antianginal effect. Although most patients probably will get the advice, when consulting a physician for angina, to maintain a certain physical activity, it cannot be assumed that they all will actually increase this activity when receiving effective treatment. We therefore consider the severity index, calculated as trinitrin consumption divided by step counter values, more useful.

Yet another way of quantitating antianginal effects has been used, namely by combining them into a total response index (8). We used in this trial the nitroglycerin consumption, the subjective rating, and the step counter readings to form such an index, but omitted the bicycle testing as the results were not complete for all patients.

In order to analyse whether one of these indices is better suited than the other to elucidate differences, we tried to estimate the interrelationships between the two by using Venn diagrams. Fig. 3 shows that if the severity index is regarded only qualitatively (response–no response), it will not differentiate between two treatments giving different total response index profiles. However, even with such a crude division of the severity index as two classes, there is good accordance between the two indices (Fig. 4).

As it is burdensome and almost impossible to get a true quantitative measurement of daily activity, which involves much more than just moving the legs, it seems probable that the total response index made up of trinitrin consumption, subjective rating (including detailed rating of common individual activities over the treatment period) and casual exercise testing is a more practical quantitative index.

Finally, a word about the influence of the climate. The seasonal changes are quite pronounced in Tampere, but we consider the possible influence of this to have been eliminated as far as possible in a trial of this sort by introducing the patients into the study over a period of 7 months and having a trial period of about 6 months (excluding the pre-run-in period). The total duration of the trial was about 14 months with start and finish in the winter. Also, the climatic influence is probably not a simple direct one, but interwoven with, e.g., the type of patient activity and the necessity for him to be in the open air, as pointed out by Aubert et al. (7).

Therapeutic effects of alprenolol and isosorbide dinitrate

ISD and, in fact, all so-called long-acting nitrates and similar compounds with a pharmacological action of dilating unchanged coronary vessels, are of questionable value in angina pectoris (12). Early uncontrolled studies, using such methods as attack counting only, commonly report positive results (24, 25, 28). In view of the well known placebo effect in angina pectoris treatment, it is not surprising to find later experience in controlled studies with negative results (3, 4, 5, 14, 17). However, there are also well controlled studies including exercise tests, both with oral ISD (10 mg q.i.d.) (8), and 5 mg sublingually (2), which show a positive result for ISD. In the present study the effect of ISD on nitroglycerin consumption was insignificant, and not larger than that of placebo (Table III). The severity index changes in the ISD group during the run-in period, however, indicate a slight effect (Table VI). This is paralleled by a reduction of systolic BP (Table VII). Whatever the cause of this fall in systolic BP, it may have contributed to the improvement of the severity index, since the BP in the ISD group was higher than in the placebo group in the pre-run-in period. In the exercise tests ISD produced a noticeable increase of exercise tolerance in only 1 patient out of 11. Thus we cannot exclude the possibility that some patients have a beneficial effect of oral ISD.

It is possible that the mechanism of action for ISD (and other long-acting nitrates) may be other than the apparent one of vasodilation. Several authors (2, 8) ha e found an increase in exercise tolerance after ISD *without* a concomitant de crease of the pressure rate product. This product has recently been shown by Holmberg et al. (19) to be well correlated to myocardial oxygen consumption in anginal patients on low and mode rate work loads. There may thus be possibilities of a true synergism between, e.g., ISD and β-blockers, as ad ocated by Russek (21) However it must be pointed out that in studies showing a synergistic effect of the two types of drugs, it has not been proven that corresponding results could not have been obtained by giving either drug alone in sufficient dosage. This ought to be established in view of the fact that the optimal dose of β-blockers may vary considerably (1 23)

From Table VI it can be seen how the severity index in cross-over periods for all patients was significantly more depressed on ALP than on ISD- the latter drug does not seem to have very much effect at all.

The results of the exercise tests (Table IX) and the total response index also demonstrate that, whereas ISD is not significantly better than placebo, ALP is more effective. The positi e ef fect of ALP in this study is in accordance with the findings of several authors (2, 6, 7 9 10 18, 22, 23) but, as with propranolol, for example (4 17) negative results with the same dosage have also been obtained (30). This may in part be due to too low a dosage, and in this trial the patients might hav done better with a higher dose since HR at 4 min on 300 kpm/min only decreased by 6% on a erage, whereas other investigators ha found a larger reduction of exercise HR under similar conditions (2, 6, 22, 23 30). However the reduction of systolic BP during work seems to have been large enough to contribute substantially to the decrease of the heart rate-systolic blood pressure product, as seen in Table IX

CONCLUSIONS

Isosorbide dinitrate, in a dose of 20 mg daily given *orally* possibly has a slight and transient beneficial antianginal effect, which may be re lated to a fall in systolic BP Exercise tolerance increased appreciably in only 1 patient out of 11 Headache was a significant side-effect.

Alprenolol, in a daily dose of 400 mg orally is significantly more effective than oral ISD both in reducing the severity of angina and in improving exercise tolerance. Exercise tolerance increased in 6 of 7 patients by 20–50% (mean 40%). No significant side-effects were seen.

A placebo medication reduced nitroglycerin consumption by about 25% over 10 weeks, but did not appreciably affect the severity of angina or the exercise tolerance.

Three out of 17 patients (18%) did not respond to any of the treatments mentioned.

The use of the quotient nitroglycerin consumed/daily activity as a severity index of angina pectoris provides a quantitative method of measuring treatment efficacy over prolonged pe riods.

Conventional ariables, such as nitroglycerin consumption, subjective rating and casual exercise testing, which alone provide useless or only par tially useful quantitative measurements, may be used together to form a total response index" capable of having a quantitative value for measuring the efficacy of treatment in angina pectoris patients over prolonged periods.

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Acta med. scand. Vol. 193 pp. 293–297 1973

THE GASTROINTESTINAL ABSORPTION OF DIGOXIN IN SEVEN PATIENTS WITH GASTRIC OR SMALL INTESTINAL RECONSTRUCTIONS

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Abstract. Digoxin-12α-³H and polyethylene glycol (PEG, unabsorbable marker) have been given orally to 5 patients (nos. 1–5) with partial gastrectomy, one patient (no. 6) with vagotomy and plastic reconstruction of the pylorus, and one (no. 7) with jejunocolostomy. A slight to moderate steatorrhoea was found in 5 patients (nos. 2–5 and 7). The ratio between the radioactivity/mg PEG in intestinal aspirates and that of the test solution demonstrated that about 55% of the label was absorbed in the upper gastrointestinal tract of patients 1–5. This value as well as those recorded for blood levels and cumulative excretion of radioactivity in the urine were within the limits previously encountered in healthy control subjects. Although not indicated by the blood levels of label, the absorption of radioactivity appeared to be slightly impaired in patients 6 and 7.

Recent studies with an isotope technique (2) have shown that the main uptake of orally administered digoxin in healthy subjects is localized to the most proximal part of the small intestine. Heizer et al. (9) evaluated the gastrointestinal absorption of the glycoside by measuring the serum levels with the use of radioimmunoassay. Digoxin was found to be poorly absorbed in several patients with malabsorption. The aim of the present investigation was to study the uptake of orally administered digoxin in patients with surgical reconstructions within the upper part of the gastrointestinal tract. It was thought, in view of the previous findings in healthy subjects, that such changes in the anatomy of the digestive tract might influence the absorption of the glycoside.

MATERIAL AND METHODS

Patients

Seven patients, 50–71 years of age, volunteered for the investigation. Three had a Billroth I (BI) and two Billroth II (BII) partial gastrectomy. Vagotomy and plastic reconstruction of the pylorus had been performed in one patient (no. 6) while the major part of the small intestine was resected in one (no. 7). A slight to moderate steatorrhoea (more than 5 g fat in faeces/day) was observed in five of the patients (Table I). For further details see Case reports.

Case reports

Patient 1. A BI partial gastrectomy (duodenal ulcer) and cholecystectomy (cholelithiasis) were performed in 1963 and 1964, respectively. During the past 2 years he had suffered from paroxysmal auricular flutter and slight hypothyroidism. He was admitted because of tachycardia. At the time of the study there was no evidence of cardiac insufficiency, hypothyroidism or diarrhoea. Present treatment: Thyranon® (desiccated thyroid gland, Organon) and Digoxin (ACO, 0.25 mg/day).

Patient 2. A BI partial gastrectomy was performed in 1969 because of gastric ulcer. After the operation she lost weight but there was no diarrhoea or dumping symptoms. She was admitted to hospital because of a slight sideropenic anaemia (Hb 9.8 g%).

Patient 3. Gastrectomy (BII) was performed because of duodenal ulcer in 1956. After the operation the patient had dumping symptoms and since 1964 frequent attacks of sinus tachycardia. Vagotomy in 1967 and reoperation with transformation of the BII to a BI gastrectomy in 1969 were of little benefit. He was admitted because of tachycardia. Oral administration of digoxin had been ineffective.

Patient 4. A BII partial gastrectomy (duodenal ulcer) was performed in 1947. In 1969 40 cm of the jejunum was resected because of multiple intestinal neurofibroma localized to the major part of the small intestine.

Patient 5. Gastrectomy (BII) was performed in 1957 because of recurrent duodenal ulcer. After the operation there was no weight loss, diarrhoea or dyspeptic symptoms. He was admitted because of melaena, the cause of this remained unknown.

Patient 6. Vagotomy and plastic reconstruction of the pylorus (gastric ulcer) were performed in 1962. The patient was reoperated upon in 1968, when a segment of

Table I. *Basal data*

Pat. no.	Sex	Diagnosis	Age (y)	Height (cm)	Weight (kg)	Faecal fat (g/day)	Serum creatinine (mg)
1	♂	Gastrectomy (BI)	50	187	87	2.3	0.7
2	♀	Gastrectomy (BI)	52	166	50	5.6	0.7
3	♂	Gastrectomy (BI)–vagotomy	54	184	70	8.8	0.9
4	♂	Gastrectomy (BII)–partial jejunectomy	56	175	59	11.8	1.0
5	♂	Gastrectomy (BII)	60	164	62	7.2	0.8
6	♂	Vagotomy–pyloroplasty	52	172	65	4.7	1.0
7	♂	Jejunocolostomy	71	170	56	32.0	1.0

the upper jejunum was removed and reinserted in the reverse direction in an attempt to cure his severe diarrhoea. This operation had little effect on the symptoms. On admission he had diarrhoea but no evidence of liver disease, sprue or pancreatic insufficiency.

Patient 7. Auricular fibrillation for many years. The distal jejunum, the ileum (altogether 4 m of the small intestine) and the ascending colon were resected in 1969 because of occlusion of the superior mesenterial artery and jejunocolostomy was applied. After the operation he lost weight, developed diarrhoea and a marked steatorrhoea.

Experimental procedures

The patients were provided with a single- or double-lumen polyvinyl tube inserted through the nose 1 2 days before the experiments (10). The position of the tube in the intestine was controlled by X-ray.

^{3}H-digoxin (0.25 mg, 50 μCi) and 5 g polyethylene glycol (PEG, unabsorbable marker) dissolved in 50 ml water followed by 100 ml water were given orally to the patients, who had fasted overnight. Intestinal aspirates (1–4 ml) were drawn from different levels during the first 3 hours. Cholecystokinin (37.5 Ivy units) was injected intravenously in most instances at 4, 7 10 and 24 hours to maintain similar experimental conditions to that in a previous study of healthy subjects (2). Blood samples were drawn at 5 10 15 20, 30, 45 60 and 90 min and at 2, 3, 4, 5 6, 7 10 and 24 hours. Urine and faeces were collected for 7 days. Total radioactivity was analysed in all specimens. PEG and pH were determined in most samples of gastrointestinal aspirates. The label in some specimens of gastrointestinal aspirates and urine was fractionated by solvent partition and thin layer chromatography (TLC).

Absorption of radioactivity in the upper gastrointestinal tract

The uptake of label was calculated by measuring the mean ratio

cpm/mg PEG in the aspirates

cpm/mg PEG in the test solution

(A/T ratio) of series of aspirates. Such calculations were performed only for aspirates in which the concentration of PEG exceeded 1 mg/ml. PEG was analysed according to the method of Hydén (11).

Determination of radioactivity and faecal fat

Aliquots of gastrointestinal aspirates (0.1 ml) and urine (1 ml) were directly pipetted into counting vials containing 15 ml of the scintillation liquid described by Bray (7). Faeces were homogenised in water. One aliquot of the homogenate was lyophilized and the dry powder analysed for radioactivity using modified Schöniger technique (13). Fat was determined on another aliquot by the method of Van de Kamer et al. (12). Plasma specimens (1 ml) were dissolved in 10 ml of an emulsifier (Insta-Gel, Packard). All determinations of radioactivity were performed with a liquid scintillation spectrometer (Packard Tricarb 3003). Quenching was corrected for by internal standardization.

Material

Digoxin-12α-^{3}H (4 Ci/mM) was obtained from New England Nuclear Corp., Boston, Mass., USA. The label was purified on the day before each experiment by TLC, using benzene/ethanol (7 3 /) as solvent system (8). The label with the same Rf value as that of digoxin was recovered by subsequent extractions with ethanol of the gel that had been scraped off the plate. Upon refractionation in system A (Table II), more than 99% of the label was recovered within the same segment as digoxin.

Non-labelled digoxin was supplied by ACO, Stockholm. Digoxigenin-bis-digitoxoside, digoxigenin-mono-digitoxoside and digoxigenin were obtained from Boehringer/Mannheim, Germany. Cholecystokinin was manufactured by the Gastrointestinal Hormone Research Group, Chemistry Department, Karolinska Institutet, Stockholm. PEG (mol.wt. 4 000) was purchased from Kabo, Stockholm.

Fractionation of radioactivity

Some samples of intestinal aspirates and urine were extracted three times with 2 vol chloroform (2). The radioactivity that accumulated in the chloroform phase was fractionated by TLC (system A or B, Table II). The gel corresponding to the site of each of the reference substances digoxin, digoxigenin and its derivatives with one or two digitoxoside groups was scraped off separately and

Table II. *Solvent system for TLC**

		Rf values			
System	Solvents (v/v)	Digoxin	Digoxigenin-bis-digitoxoside	Digoxigenin-mono-digitoxoside	Digoxigenin
A (ref. 14)	Cyclohexane acetone acetic acid (49 49 2)	0.28	0.33	0.39	0.45
B (ref. 1)	Ethyl acetate: n-butyl-alcohol (90 10)	0 28	0 38	0.52	0 60

All chromatographies were performed on glass plates (20 20 cm) covered with 250 μ silica gel (Merck F 254).

transferred into separate glass-stoppered tubes and extracted with 8 ml Bray scintillation liquid. Aliquots of this solution were pipetted into counting vials and analysed for radioactivity

RESULTS

The A/T ratios recorded for aspirates collected 80–95 cm from the nose indicated that 34–65 (mean 46) % of the label had been absorbed in the 5 patients (nos. 1–5) with partial gastrectomy (Table III). More distally (110–130 cm from the nose) the cumulative uptake averaged 53 (34–62) %

The absorption of radioactivity appeared to be lower in patients 6 and 7 The mean uptake for patient 6 was calculated at 30% when the major part of the PEG had reached the upper jejunum 130 cm from the nose. The corresponding result for patient 7 at 90 cm was about 30%

The patient also tended to differ with regard to the amount of label eliminated in the urine. The cumulative excretion in patients 1–5 averaged 25.5 (18.4–28.8) after 1 day and 50.9 (39 4–61 0) % after 7 days. The corresponding values for patients 6 and 7 were 18.2 (16.7 19 7) and 35.1 (26.6, 43 6) % respectively The mean excretion of radioactivity in the faeces in patients 1–5 was 31.9 (21.3–39 0) % The low values encountered in the faeces of patients 6 and 7 were in part due to incomplete collection of faeces (Table IV).

The plasma level of label was approximately the same in all patients. When the peak level was observed, the total plasma volume contained 1.5–2.2% of the given dose (Table IV). At 4 hours the corresponding values were 0.1–0.4%

Four samples of gastrointestinal aspirates were further analysed. All label was extractable with chloroform and 97–98% of the total radioactivity appeared to be attached to digoxin (Table V). Non-chloroform extractable radioactivity (1–13%) was encountered in 5 of the urine specimens analysed (Table VI). Digoxigenin and its derivatives with 1–2 digitoxose groups accounted for 0–9 (mean 5) % of the total radioactivity

Table III. *A/T ratios and pH of gastrointestinal aspirates*

Pat no.	GI aspiration distance from nose (cm)	GI aspirates Time of collection (min)	No.	A/T ratio (mean)	A/T ratio (range)	pH of aspirates (mean)
1	80	10–30	4	0.64	(0.60–0.68)	7 3
	110	10–36	5	0.43	(0.34–0.51)	7 1
2	80	10–23	3	0.46	(0.32–0.54)	7 9
	130	13–53	6	0.38	(0.26–0.54)	7.3
3	80	12–90	7	0.58	(0.49–0.77)	6 8
	110	50–105	4	0.40	(0.22–0.51)	6 6
4	55	5–110	15	0 61	(0.32 1.06)	7 1
	95	13–151	9	0.35	(0 18–0.62)	7 9
5	80	10–29	6	0.66	(0 51–0 79)	—
	110	5–29	6	0 66	(0.45–0.90)	7 0
6	100	3–25	7	0 68	(0.56–0 78)	7 4
	150	3–25	6	0.70	(0 61–0 80)	7.5
7	90	33–165	11	0.72	(0 56–0 84)	6 5

Table IV *Radioactivity in total plasma volume, urine and faeces in % of administered dose*

Pat. no.	Plasma: Peak level (%)	(min)	At 24 h (%)	Urine 1 d.	0–7 d.	Faeces (0–7 d.)	Urine and faeces (7 d.)
1	2.2	30	0.4	28.8	53.1	21.3	74.4
2	1.7	45	0.1	28.1	61.0	23.5	84.5
3	1.6	45	0.3	24.6	49.6	39.0	88.6
4	1.9	20	0.2	27.5	51.2	38.6	89.8
5	1.5	25	0.1	18.4	39.4	37.1	76.5
6	1.6	45	0.2	19.7	43.6	14	—
7	2.1	125	0.2	16.7	26.6	26[a]	—
3 healthy controls (2)	1.5 (1.3–1.9)	30–180	0.3 (0.2–0.4)	21.3 (20.1–23.7)	46.7 (40.5–56.7)	37.9 (14.9–52.3)	84.6

[a] Incomplete collection.

DISCUSSION

The gastrointestinal absorption of radioactivity following oral administration of ³H-digoxin to healthy subjects was found to be about 60% (2). During exposure to the acid gastric content a minor part of the glycoside was hydrolysed, which resulted in the formation of digoxigenin and its derivatives with one or two digitoxose groups. The uptake of radioactivity was approximately the same in subjects given ³H-digoxin and ³H-digoxigenin. Accordingly the absorption of radioactivity after administration of the former compound appears to reflect the combined uptake of the glycoside and its metabolites produced in the upper gastrointestinal tract. Under such conditions the total absorption of intact digoxin in healthy subjects should be approximately 50%, the main uptake being localized to the duodenum and the upper jejunum.

In the present 5 patients with partial gastrectomy the absorption of radioactivity appeared to be as effective as that previously observed for healthy subjects. The A/T ratios of the gastrointestinal aspirates, the blood levels and the urinary output of label were within the same range in both groups. The cumulative urinary excretion of radioactivity which represents the minimal absorption of label, averaged 51 (39–61) % in patients 1–5 and 47 (41–57) % in the healthy subjects. The uptake of the intact glycoside may be even higher in the patients since, in contrast to the controls, there was little decomposition of the label in the aspirates analysed. In the healthy subjects 0–6% of the radioactivity in the stomach, duodenum and jejunum was at

Table V *Fractionation of radioactivity in gastrointestinal aspirates (% of total radioactivity)*

Pat. no.	Level of aspiration (cm)	Time of collection (min)	Extractable with chloroform	Distribution upon TLC: Digoxin	Digoxin metabolites[a]
4	55	45	100	98	1
	95	13	100	98	0
5	80	16	100	97	1
	110	15	100	97	2

[a] Digoxigenin-bis-digitoxoside, digoxigenin-mono-digitoxoside and digoxigenin.

Table VI. *Fractionation of radioactivity in urine specimens (% of total radioactivity)*

Pat. no.	Time of collection (h)	Extractable with chloroform	Distribution upon TLC: Digoxin	Digoxin metabolites[a]
1	0–6	87	73	9
	12–24	93	78	8
2	12–24	96	76	3
	72–96	100	92	0
3	12–24	90	86	4
	48–72	99	93	2

[a] Digoxigenin-bis-digitoxoside, digoxigenin-mono-digitoxoside and digoxigenin.

tached to digoxigenin, digoxigenin-mono-digitoxose or digoxigenin-bis-digitoxose (2).

Although not indicated by the plasma levels of label, it appeared from the A/T ratios that the absorption of digoxin was slightly impaired in patients 6 and 7. The lowest urinary excretion of label (27 %) was likewise encountered in patient 7 who had a jejunocolostomy and in whom only a very short segment of the upper small intestine was intact.

Heizer et al. (9) studied serum levels of digoxin in patients with marked steatorrhoea due to sprue, pancreatic insufficiency, hypermotility (neoplasm, laxative) and small bowel resection. The subjects received 0.25 mg digoxin/day and steady state serum levels were reached after 6 days. Whereas the concentration in control subjects averaged 1.3 ± 0.3 (S.E.) ng/ml, the values for 8 patients with malabsorption ranged between < 0.2 and 0.7 ng/ml. The highest values (0.9–1.6 ng/ml) were encountered in 1 subject with ileal resection and 2 patients with pancreatic insufficiency.

The mechanisms of absorption of cardiac glycosides are not fully understood. Studies in rats (8) indicated that digoxin is absorbed by a passive, nonsaturable transport mechanism that does not require metabolic energy. Heizer et al. (9) suggested that the absorption of digoxin is not dependent upon the secretion of the bile salts and pancreatic juice. The major part of orally administered drugs such as digoxin (2), digitoxin (3) and several anticholinergic drugs (4, 5, 6) is absorbed in the most proximal part of the small intestine. The present findings in patients with BII partial gastrectomy demonstrate that the uptake of digoxin is unimpaired even when a very active area of absorption, i.e. the duodenal mucosa, is bypassed.

ACKNOWLEDGEMENTS

The present investigation was supported by the Swedish Research Council, the Swedish National Association against Heart and Chest Diseases and ACO Lakemedel AB.

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Acta med. scand. Vol. 193 pp. 299–302, 1973

ARRHYTHMIAS IN DIFFERENT TYPES OF ACUTE CORONARY HEART DISEASES

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Abstract. The incidence of arrhythmias has been investigated in 100 patients with intermediate coronary syndrome (ICS), in 42 with small acute myocardial infarction (AMI) and in 147 with frank AMI. A continuous ECG has been monitored from admission until at least 48 hours after the onset of symptoms. Bradyarrhythmias, atrial flutter and fibrillation and short-lasting ventricular tachycardia were significantly more often seen among patients with small and frank AMI than among patients with ICS. Complete AV block was seen in one patient with small AMI, otherwise no patients with small AMI or ICS had AV block, ventricular tachycardia or fibrillation. Serious arrhythmias did not start more than 48 hours after onset of symptoms in patients with small AMI or ICS as opposed to patients with frank AMI. Serious arrhythmias were more often seen in patients with pre-existing CHD than in patients without. The results suggest that the observation period in monitored beds can be short, and in case of need omitted, without taking any great risks in patients with small AMI and ICS. This is in contrast to patients with frank AMI, who certainly need these beds and often for more than 48 hours.

The development of techniques for continuously monitoring the ECG has revealed a very high incidence of arrhythmias in acute myocardial infarction (AMI) (2). Factors in the pathogenesis of these arrhythmias are not only myocardial necrosis, but also ischemia involving pacemakers and conduction pathways as well as increased sympatho-adrenal discharge and vasovagal cardiac reflexes (5). Arrhythmias may therefore be seen also in acute coronary heart diseases (CHD) with small or no myocardial necrosis. A relationship between the site of AMI and the type of arrhythmias has been found (5), and a greater incidence during the first hours after onset has been demonstrated (4). The incidence of arrhythmias in patients with small AMI and with intermediate coronary syndrome (ICS) is, however uncertain. The importance of pre-existing CHD is also unknown in this connection. In the coronary care units (CCU) there is sometimes a shortage of monitored beds. It is therefore important to know the incidence of arrhythmias in different categories of acute CHD.

MATERIAL AND METHODS

The analysis is based on a consecutive series of 100 patients with ICS and 189 cases with AMI admitted during the same period. The patients were seen over a period of 20 months at the CCU at Diakonissehusets Hospital, Oslo. Only patients admitted to the unit within 4 hours after the onset of symptoms were included. A continuous ECG was monitored in all patients until at least 48 hours after the onset of attack. A 4-channel oscilloscope was continuously observed and all arrhythmias recorded. A 12-lead ECG and laboratory tests were taken immediately after admission and daily on the following 4 days.

A diagnosis of ICS was made if all the three following criteria were fulfilled.

1. A clinical history with typical chest pain of more than 15 min duration.

2. Transient ST T changes in the ECG in connection with the attack. In patients with verified pre-existing CHD, who described the present attack as typical, ST T changes were not required.

3. Absence of the ECG and laboratory signs of myocardial infarction mentioned below.

A diagnosis of AMI was made from one of the following two criteria.

1. Significant Q and QS changes in the ECG (1).

2. A clinical history of chest pain connected with SGOT > 50 U and SCPK 120 U and serial ST T changes in the ECG. In patients with verified pre-existing CHD ST T changes were not required.

The AMIs were called *small* if serial Q-QS changes were absent, maximal SGOT < 120 U and maximal SCPK < 300 U. The remaining AMIs were called *frank*.

The average age of the patients with ICS was 62.8 years and of the patients with AMI 64.0 years. The male female ratio was 3.7 4.1. There was no difference between the two groups in the time from onset of symp-

Table I. *Incidence of bradyarrhythmias in acute CHD*

	AMI ( )		ICS
	Frank (N = 147)	Small (N = 42)	(%) (N 100)
Sinus bradycardia, sinus arrest, junctional rhythm	23.8	23.8	10
Partial AV block	4.1		
Complete AV block	4.8	2.4	

toms to arrival in the CCU. Exactly the same policy was followed in the treatment of the patients in the different groups.

RESULTS

In the patients with AMI some kind of rhythm disturbances occurred in 96.3%. Sporadic ventricular extrasystoles and supraventricular extrasystoles were seen as the only arrhythmia in 20.1%. The remaining 76.2% had more important arrhythmias. In patients with ICS important arrhythmias were seen in 37%. The different types of arrhythmias are reported below.

Bradyarrhythmias (Table I). Sinus bradycardia (< 50/min), sinus arrest (or block) and junctional rhythm were often seen together in the same patients and are combined here. The incidence of these arrhythmias was significantly greater in patients with AMI than with ICS ($p < 0.01$). When occurring in patients with AMI a diaphragmatic localization was seen significantly more often than an anterior one.

Table II. *Incidence of ventricular arrhythmias in acute CHD*

	AMI ( )		ICS
	Frank (N 147)	Small (N 42)	( ) (N 100)
Accelerated idioventricular rhythm	5.4	4.8	
Extrasystoles 1 in 10 as the only arrhythmia	29.9	26.2	20
Short-lasting ventricular tachycardia[a]	20.5	21.4	8
Ventricular tachycardia[b]	8.1		
Ventricular fibrillation[b]	6.1		
Multiform or multifocal extrasystoles	44.2	19.0	14

[a] Three or more successive beats of 30 sec duration.
[b] And one or more of the arrhythmias mentioned above.

Table III. *Incidence of supraventricular arrhythmias in acute CHD*

	AMI (%)		ICS
	Frank (N = 147)	Small (N = 42)	( ) (N = 100)
Supraventricular extrasystoles only	34.7	23.8	31
Supraventricular tachycardia	5.4	4.7	
Atrial flutter or fibrillation	13.6	16.7	7

In patients with frank AMI partial AV block was seen in 4.1%. In addition 4.8% had complete AV block with or without preceding partial block. Complete AV block was also seen in 2% as an agonal event. One patient (2.4%) with a small AMI had an uncomplicated complete AV block with a heart rate not below 60/min. No patients with ICS developed AV block, but one had chronic partial block.

Ventricular arrhythmias (Table II). Accelerated idioventricular rhythm with heart rate between 60 and 100/min was seen in only about 5% of patients with AMI, but was not observed in patients with ICS. Parasystoles were not seen at all, whereas extrasystoles were seen very frequently in all groups. Short selfterminating paroxysms of ventricular tachycardia were seen significantly more often ($p < 0.01$) among patients with AMI than among patients with ICS. All 8 patients with ICS and short ventricular tachycardia had less than 10 successive ectopic beats. Ventricular tachycardia and fibrillation were never seen in patients with small AMI or ICS. In patients with frank AMI the incidence of ventricular fibrillation was 6.1%. In 2 patients (1.4%) the ventricular fibrillation was primary. Four patients (2.8%) had ventricular fibrillation as an agonal event and they have not been included.

Multiform or multifocal extrasystoles were seen in 44.2% of patients with frank AMI. This was significantly more frequent ($p < 0.01$) than in the other two groups.

Supraventricular arrhythmias (Table III). Extrasystoles were frequently seen and with the same incidence in the different groups. Tachycardia (atrial and junctional) was seen in about 5% of patients with AMI and not at all in ICS. Atrial

flutter and fibrillation, often of short duration, were seen in about 15% and 7% of patients with AMI and ICS, respectively. In addition 3.4% of the patients with AMI had pre-existing atrial fibrillation.

In Table IV the most important arrhythmias starting more than 48 hours after the attack are listed. It is seen that no arrhythmias were observed among patients with ICS. One patient with a small AMI had transient atrial fibrillation. Among patients with frank AMI 14 died within 48 hours after the attack. Among the remaining patients both transient atrial fibrillation, ventricular tachycardia and partial AV block were seen. Seven patients (5.3%) developed ventricular fibrillation, in 3 cases it was primary. One of the latter had only sporadic extrasystoles during the first 48 hours, while the others had had multifocal extrasystoles and short episodes of ventricular tachycardia.

The incidence of arrhythmias in patients with and without pre-existing CHD is seen in Table V. Only arrhythmias for which a difference between the two groups was found have been listed. It is seen that, among patients with AMI, arrhythmias are seen more often in those with pre-existing CHD. In patients with ICS and without known pre-existing CHD atrial fibrillation or flutter was never seen, as opposed to patients with pre-existing CHD.

DISCUSSION AND CONCLUSION

In the present study acute CHD has been graded into ICS, small and frank AMI. A division into these groups may seem artificial, and boundary cases certainly exist. From a practical point of view it is useful, however, since the prognosis in these groups is different. The terms which have been used to describe the condition intermediate between angina of effort and AMI have been many, including pre-infarction syndrome (7), acute coronary insufficiency (8), impending myocardial infarction (3) as well as ICS (6).

AMIs with the smallest ECG changes and the lowest enzyme values have been called small. This condition is often called subendocardial or non-transmural infarction. In some cases it will be impossible to differentiate between this condition and ICS. The grouping of patients will therefore sometimes depend upon more or less arbitrarily chosen definitions. Concerning the incidence of arrhythmias the results in the present study have shown that small AMIs stand between the ICSs and frank AMIs.

In a CCU all the above mentioned types of acute CHD are often seen. In the present study comprising 289 cases, 50.9% were considered to have frank AMI, 14.5% small AMI and 34.6% ICS. The relative proportion of these groups will depend upon the criteria used and upon the admission policy. In our hospital we have an out patient department for admission of patients suffering from chest pain. A comparatively high incidence of ICS is therefore probably seen in our CCU.

The results concerning frank AMI are in good accordance with earlier studies (2). Only very few patients (3.7%) had no arrhythmias. In 20.1% only mild arrhythmias were seen, while in the remaining 76.2% the arrhythmias necessitated closer observation and medical treatment. Opposed to this are the results found among patients with small AMI and ICS. One patient with small

Table IV *Incidence of arrhythmias starting >48 hours after the onset of acute CHD*

	AMI ( )		ICS
	Frank (*N*=133)	Small (*N*=42)	( ) (*N*=100)
Atrial fibrillation	1.5	2.4	
Partial AV block	1.5		
Ventricular tachycardia	2.3		
Ventricular fibrillation	5.3		

Table V *Incidence of arrhythmias in patients with and without pre-existing CHD*

	AMI ( )		ICS ( )	
	First attack	Pre-existing CHD	First attack	Pre-existing CHD
Atrial flutter or fibrillation	14.1	18.6		10.6
Short ventricular tachycardia	18.5	22.7	2.9	10.6
Ventricular tachycardia	8.6	7.6		
Ventricular fibrillation	6.5	10.3		
Complete AV block	2.2	8.3		

AMI had transient AV block, otherwise no patients had AV block, ventricular tachycardia or ventricular fibrillation. Twelve patients (28.6%) with small AMI were given lignocaine and 3 patients (7.1%) atropine. Of the patients with ICS 11 (11%) were given lignocaine and 2 (2%) atropine. This prophylactic treatment may have *prevented more serious arrhythmias. The treatment* could, however, be discontinued after 1–4 hours without relapse. Most probably the chances of developing serious arrhythmias are small even without prophylactic treatment.

Patients with small AMI and ICS were often not monitored for more than 48–72 hours after the onset of the attack. Mild and short-lasting arrhythmias may therefore have been overlooked. Severe arrhythmias have certainly not been overlooked, since the patients were still in the CCU. While both severe and fatal arrhythmias started more than 48 hours after the onset of symptoms among patients with frank AMI, they were not at all seen in patients with small AMI and ICS.

The incidence of arrhythmias was higher in patients with pre-existing CHD than in those without. The difference was greatest concerning the most serious arrhythmias such as complete AV block and ventricular fibrillation. This is not surprising: other complications such as heart failure and cardiogenic shock are also more frequent among patients with pre-existing CHD.

The results in the present study point to the conclusion that patients with the smallest AMI seldom have serious arrhythmias, and patients with ICS probably not at all. The observation period in monitored beds can therefore be short and, in case of need, omitted without taking any great risks. This is in contrast to patients with *frank AMI, who certainly need these beds and* often for more than 48 hours.

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Acta med. scand. Vol. 193 pp. 303–306, 1973

DIAGNOSTIC PERCUTANEOUS SUPRASTERNAL AND LEFT VENTRICULAR PUNCTURE OF THE HEART AND GREAT VESSELS

Indications and Complications

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Abstract. In order to evaluate indications, complications and factors which may influence the rate of successful examinations, 159 suprasternal and left ventricular punctures performed in 139 patients with organic heart diseases have been analysed. In suprasternal puncture sufficient pressure readings were obtained in 86% of the examinations, whereas pressures from the left ventricle were obtained in 79% by left ventricular puncture. In combined suprasternal and left ventricle puncture the rate of success was 70%. The main indication for combined suprasternal and left ventricular puncture is found to be aortic stenosis or combined mitral and aortic stenosis. Distinct apex beat, counterclockwise rotation in ECG and enlargement of the left ventricle on X-ray increased the rate of success, whereas functional capacity, age, heart rhythm and other ECG findings were found to be of no importance. Only three major complications occurred, all pneumothoraces which necessitated surgical treatment; none were fatal. Severe congestive heart failure, emphysema, anticoagulant therapy or tendency to bleed are regarded as relative contraindications.

Percutaneous suprasternal puncture (SP) was first described by Bjork et al. (1) in 1953 and Radner (7) in 1954 and left ventricular puncture (LVP) by Nuvoli (5) in 1936 and later on by Ponsdomenech and Nunez (6). Suprasternal puncture has been used routinely in our laboratory since 1956 and the combined SP and LVP examination since 1958. During this period more than 750 examinations have been performed (3, 4). In a cooperative study on cardiac catheterizations (2) based on more than 12 000 hemodynamic investigations, SP was not used and LVP was performed only in 460 cases.

In spite of new catheterization techniques for hemodynamic investigations of the heart and the great vessels, we find there still remains a group of patients in which SP and LVP are useful. The purpose of this paper is to describe indications, complications and factors which may influence the rate of successful examinations.

MATERIAL AND METHODS

A retrospective study was undertaken of all SP and LVP performed in the period 01.04.1963–31.12.1971. In 139 patients 159 examinations, 115 combined SP and LVP, 38 SP and 6 LVP were performed. One patient was examined four times, one three times and 8 patients twice during the same admission because of failure of the first examination. The indication has been suspicion of left-sided valvular heart disease.

The techniques and procedures used are the same as described in detail by Tybjærg Hansen et al. (2, 3), except for the premedication, which has been changed from 200 mg phenobarbitone and 50 mg pethidine chloride intravenously to 75 mg pethidine chloride given intramuscularly half an hour before the investigation is started. An X-ray of the chest in antero-posterior projection was taken before and just after the examination. The procedure in combined SP and LVP was recorded as successful when the gradient across the ostium corresponding to the suspected valvular lesion was obtained.

Age and sex distribution is seen in Fig. 1 and final diagnoses in Fig. 2. "Other combinations of valve diseases" include 4 patients with combined aortic, mitral and tricuspid valve disease, and 1 with valvular pulmonic stenosis. In 15 patients, in whom no valvular heart disease was found, the diagnosis was arteriosclerotic heart disease in 8 cases, cardiomyopathy in 4, and ventricular septal defect, *primary pulmonary hypertension and persistent* duct combined with coarctation of the aorta in 1 patient each.

The functional capacity according to the classification by the American Heart Association was as follows: Class I 8 patients, II 58 patients, III 64 patients and IV 9 patients.

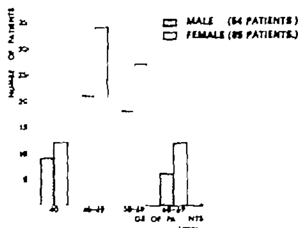


Fig 1 Age and sex distribution in the 139 patients examined.

RESULTS

The percentage of successful SP and LVP together with a successful combined SP and LVP examination is shown in Table I. In 34 cases in which the combined SP and LVP examination was regarded as unsuccessful, both SP and LVP failed only in 12 cases, one of which was caused by equipment failure. In 6 of the unsuccessful cases, 3 SP and 3 LVP, sufficient pressure information was obtained in a new examination performed by a more experienced investigator.



Fig. 2. Diagnoses in the 139 patients examined.

Table I. Number of SP, LVP and combined SP and LVP

Percentage of total number of punctures in each group given within parentheses

Type of puncture	No. of punctures Total	Successful	Unsuccessful
SP	153	131 (86)	22 (14)
LVP	121	96 (79)	25 (21)
Combined SP and LVP	115	81 (70)	34 (30)

In 129 SP information was available about the number of attempts performed in each examination. The examination was successful in 60% of cases after one attempt. In 48 examinations 2 to 4 attempts were made. The examination was successful in 50% of cases at the second, third and fourth attempts, respectively.

In 99 LVP information was available about the number of attempts performed in each examination. The percentage of successful examinations was 63% when only one attempt was performed. In 15 patients 2 or 3 attempts were necessary to obtain sufficient pressures. In these cases only 33% of the examinations were successful at both the second and the third attempts. The following factors were analysed and found to influence the rate of success.

Diagnosis. In 25 patients with pure mitral stenosis pressure from the left ventricle was obtained in 14 patients (56%). In 53 patients with aortic stenosis successful LVP was performed in 46 patients (87%). When SP was applied in 93 patients with mitral stenosis, left atrial pressure was obtained in 79 patients (85%) whereas the left atrium was only punctured in 36 patients among 60 without mitral stenosis (60%). In 49 patients with aortic stenosis, pressure from the aorta was obtained in 47 patients (96%). In 104 patients without aortic stenosis successful examination was performed only in 91 (87%).

ECG. In 8 patients with counterclockwise rotation ($R > S$ in V_3) LVP was successful in all cases. In 31 patients with clockwise rotation ($S > R$ in V_4) LVP was successful in 21 (68%). With intermediate position of the heart in the

horizontal plane the rate of success was 79% in 82 patients.

X-ray of the chest. LVP was sufficient in 76 of 89 patients (85%) when X ray showed enlarged left ventricle and in 20 of 32 patients (63%) with normal left ventricle.

Apex beat. In 100 patients in whom the apex beat was distinct, LVP was successful in 83. In 11 patients in whom the apex beat was difficult to localize, pressure from the left ventricle was only obtained in 6 (55%) In 3 patients the apex beat could not be localized, and in none of these was a pressure measurement obtained.

The following parameters were analysed and found to be without influence on the rate of success: functional capacity age, heart rhythm, left ventricular hypertrophy and rotation of the heart in the frontal plane in ECG and size of the left atrium on X-ray

Table II. *Complications after SP LVP and combined SP and LVP*

Complications	Type of examination: Combined SP and LVP	SP	LVP
Major complications			
Severe left-sided pneumothorax	3	—	—
Minor complications			
Slight left-sided pneumothorax	6	—	—
Small mediastinal hematoma (X-ray)	6	1	—
Vasovagal reactions	4	—	—
Hemoptyses	—	1	—
Pleural effusion	2	—	—
Total (in 23 pats.)	21	2	—
Total no. of examinations	115	38	6

Complications

In 150 examinations (Table II) no fatal complications occurred. Three patients, one of whom had emphysema of the lungs, had severe left sided pneumothorax which necessitated surgical drainage. Six patients with slight left-sided pneumothorax had only few or no symptoms, and the pneumothorax regressed spontaneously

In 6 patients X-ray of the chest after the examination gave suspicion of small mediastinal hematoma None of them had complaints, except one with slight chest pains, and the X-rays were normalized in few days. Four cases of vasovagal reactions were transient and i.v injection of atropine was necessary only in one case with immediate relief of the symptoms. The patient with hemoptysis expectorated 15 ml blood immediately after the puncture needle was introduced, but there was no other complaint and the X ray of the chest was normal. Two patients had a slight left-sided pleural effusion on the control X ray There were no symptoms and the effusion disappeared within one week. In one patient a pleural friction rub was heard after the examination. X-ray of the chest was normal, and stethoscopy of the lungs normalized in a few days. In three patients a coronary artery was punctured during LVP· none of them had any complaints.

Concerning factors which might influence the frequency of complications, it should be mentioned that prior thromboembolic accidents, which were seen in 26 patients, did not influence the rate of complications. There was no correlation between complications and ECG findings, X-ray of the chest, diagnosis, functional capacity earlier anticoagulant therapy or age.

On the other hand it was of importance that the apex beat could easily be localized. In 100 patients in whom the apex beat was distinct there were only 16 minor complications, whereas 5 of 14 patients without distinct apex beat developed pneumothorax. Three of these cases were severe. When the apex beat was difficult to localize more puncture attempts were used in each examination and the rate of complications increased.

DISCUSSION

Neither in the American Heart Association's cooperative study on cardiac catheterization (2) nor in previously published papers from our laboratory (3 4) were factors analysed which may influence the rate of success or complications. SP of the great vessels and left atrium may be performed with low complication rate, as shown by Tybjerg Hansen et al. (3) as only one major complication was reported in 500 examinations.

In another publication (4) from our department major complications occurred in 8 of 149 examinations when combined SP and LVP was performed. The same type of complications with an incidence of 2–3% as found in our material were

reported by Braunwald (2) who used only LVP These facts point to LVP as the main cause of the major complications. In our material 5 of 9 patients who developed pneumothorax, severe in 3 occurred among 14 patients in whom the apex beat was not distinct. Therefore it must be stressed that LVP should only be used when the apex beat is easy to localize. Other contraindications are chronic pulmonary diseases, especially emphysema of the lungs (3), severe congestive heart failure (4), and disturbances in the bleeding factors including anticoagulant therapy (2). The fact that the frequency of major complications in this study was lower and the complications less severe than in a previous study from our department (4) might be explained by a change in the premedication and a reduction in the duration of the examinations. In this study the combined SP and LVP examination was carried out within 10–15 min. This was achieved by abstaining from cardiac output determinations, which were previously included in the examinations.

During recent years the main indication for combined SP and LVP has been patients with both aortic and mitral stenosis, as retrograde or transseptal catheterization of the left ventricle is often very difficult in this category of patients. Furthermore SP and LVP have been useful in patients in functional class IV who are unable to remain in supine position for more than a quarter of an hour

As shown by the factors which may influence the rate of successful examinations, SP may be useful especially in patients with pure mitral stenosis, whereas the combination of SP and LVP is more suitable in patients with pure aortic stenosis or combined mitral and aortic stenosis.

CONCLUSION

The suprasternal puncture (SP) is a technically easy method for determination of pressures in the aorta, the pulmonary artery and the left atrium with a high rate of successful examinations and only very few complications. Left ventricular puncture (LVP) is more difficult to perform and should be used only in patients in whom the apex beat is distinct, as the complication rate increases considerably when the apex beat is absent. The main indication for combined SP and LVP is patients with combined mitral and aortic stenosis, in whom the examination has a high frequency of success and is accompanied by few complications. Isolated SP may be used in patients with pure mitral stenosis, whereas combined SP and LVP in this category of patients is not useful because of its relatively low rate of success. Contraindications are severe congestive heart failure, emphysema, anticoagulant therapy or tendency to bleed.

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Acta med. scand. Vol. 193 pp. 307–309 1973

CHANGES IN ANTITHROMBIN III LEVELS FOLLOWING CESSATION OF ANTICOAGULANT THERAPY

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Abstract. Antithrombin III (At-III), α_2-macroglobulin and fibrinogen have been followed for 4 weeks after cessation of warfarin treatment in 20 individuals. There were no significant changes in fibrinogen or α_2-macroglobulin levels. At-III decreased significantly in serum and in plasma. It is suggested that these changes reflect a low grade continuous coagulation which was suppressed during anticoagulant treatment.

Antithrombin III (At III) reacts with thrombin (1) and with activated factor X (3, 20), probably by the formation of inactive complexes. During coagulation in vitro, At III is partly consumed (4) and the At-III concentration in normal serum is about 65% of that in plasma (5).

An increased consumption of At-III in vivo may probably also occur in certain pathological conditions. In disseminated intravascular coagulation (DIC) the At III level is often markedly lower than normal (12, 13). In women using certain contraceptive pills the moderate reduction of the At III level seems to reflect a low degree of continuous intravascular coagulation (5, 11).

It was early observed that serum antithrombin increased during treatment with dicumarol (9, 16). The increase in serum antithrombin paralleled the decrease in plasma prothrombin level (16) and was thus another expression of the reduced in vitro coagulability of the blood during anticoagulant therapy.

Several authors state that treatment with coumarins does not influence the plasma antithrombin level (8, 10, 15, 16). In contrast, Lasch (12) and Péter (18) reported a marked increase in both serum and plasma antithrombin levels during peroral anticoagulant treatment. Hensen and Loeliger (6) found a slight increase in plasma At-III activity at the beginning of coumarin treatment. The discrepancy regarding the influence on plasma antithrombin might reflect the different assay methods used. In order to obtain more clear information, we have studied this problem by immunochemical methods, which have a higher precision than conventional coagulation methods.

The study was conducted on 20 patients who had been on long-term warfarin treatment. The drug was discontinued abruptly while the patients were considered to be in a steady state and while the coagulation activity was in the therapeutic range.

The concentration of the main thrombin inhibitor At-III, was followed in plasma and serum. The concentrations of α_2-macroglobulin (α_2M), which probably is a weak thrombin inhibitor (2, 19) and of fibrinogen were also measured.

MATERIAL AND METHODS

The patient material consisted of 4 women and 16 men, mean age 64 years (range 54–80), who had been treated with warfarin sodium for a period ranging from 2 months to 10 years, mean 4 years 10 months, because of ischemic heart disease. No thrombotic episodes were diagnosed during the 2 months prior to cessation of treatment with warfarin, nor during the observation period (4 weeks).

During treatment with warfarin a coagulation activity of 10% as measured by the thrombotest method (15), was aimed at.

Plasma. About 5 ml blood was collected into 13 × 100 mm polystyrene tube sprayed with K_2EDTA (AG Greiner, Germany). The tubes were immediately capped, turned at least 5 times to dissolve the EDTA, centrifuged at 2 000 *g* for about 30 min, and the plasma was stored at −25°C until assay. Control experiments with sodium citrate and sodium chloride solutions were performed to ascertain that the amount of EDTA used was

Table I. *Changes in coagulation activity (thrombotest), At III α_2M and fibrinogen levels after cessation of warfarin treatment (last dose taken on day 0)*

Mean ± S.D. within parentheses. For details, see Material and methods. Paired comparison was performed between the alues obtained on day 1 and the other alues

	Day 1	Day 2	Day 7	Day 14	Day 28
Thrombotest (%)	10.1 (2.47)	57.2 (30.65)	>100 (all 20)	>100 (all 20)	>100 (all 20)
At-III in plasma ( )	163.0 (20.01)	160.4 (12.14)	161.2 (19.30)	149.2 (31 72)	153.2 (17.93)
At-III in serum (%)	131.3 (18.71)	117 1 (20.82)	106.6 (15.37)	96.0 (11.28)	92.0 (14.53)
Fibrinogen in plasma	155.6 (33.22)	155.8 (32.84)	154.3 (34.23)	165.8 (61 71)	166.0 (46.22)
α_2 M	102.8 (22.91)	104.5 (25 02)	95.30 (17.60)	102 6 (22 19)	100 0 (27.81)

$p < 0.001$

sufficient to inhibit any thrombin generation and hence At III consumption after blood sampling.

Serum. Whole blood was collected into glass tubes, which ere covered with rubber stoppers for 2–4 hours at room temperature, centrifuged, and the serum kept at −25°C.

Immunochemical assays. Rabbit antiserum against At-III was obtained from Nyegaard, Oslo, Norway. Rabbit antiser against α_2M and fibrinogen were obtained from Behringwerke, Marburg/Lahn, Germany. The single radial immunodiffusion technique of Mancini et al. (14) was used. The gel buffer contained 0.005 M EDTA to avoid thrombin generation.

The results of the At III and α_2M assays were e pressed in % of standard serum (containing equal amounts of serum from 500 normal blood donors). The fibrinogen concentration was expressed in % of normal standard plasma, hich was found to contain 3.0 mg clottable protein ml.

Statistical calculation

The concentration found in the samples obtained 4 7 14 and 28 days after cessation of warfarin treatment were compared with the concentration on the day after cessation of treatment (day 1). The paired comparison (according to Student's *t*-test) was performed with the Olivetti Programma computer.

RESULTS

The thrombotest alues increased rapidly after cessation of warfarin medication (Table I). As expected, serum At III decreased and it is note worthy that it continued to decrease for more than 2 weeks. Plasma At III showed a moderate, but statistically highly significant decrease, which also seemed to continue for 2–3 weeks. There was no consistent change in the α_2M level. The moderate increase in fibrinogen concentration was not statistically significant.

DISCUSSION

Unlike earlier authors, who either have compared antithrombin in treated and untreated groups or studied changes during initiation of anticoagulant treatment, we have studied the changes occurring after *cessation* of anticoagulant treatment. We have chosen this approach for the following reasons.

Firstly we felt that studying changes within one group of individuals would give more reliable results than studying the difference between a treated and an untreated group.

Secondly anticoagulant treatment is often initiated when the patient is in a labile phase as shortly after a myocardial infarction. Under such conditions the acute phase of the disease itself as well as the warfarin medication might influence the blood values studied.

When warfarin medication was withdrawn from patients considered to be otherwise in a steady state, serum At III (as expected) showed a considerable and gradual decrease, but there was also a significant, although moderate, decrease in the plasma At III level. The decrease in plasma At III might result either from a decreased rate of pro-

duction or from an increased rate of utilization. According to present experience At-III belongs to the proteins which do not show increased values as response to, e.g., inflammatory reactions, that is, human At III does not belong to the acute phase reacting proteins.

It is possible that the warfarin medication directly or indirectly might increase the production rate of At III. In view of the findings in intravascular coagulation, however we would like to suggest that the decrease in plasma At-III after withdrawal of warfarin is the result of increased antithrombin utilization.

There is as yet no definite evidence of a continuous coagulation going on in apparently healthy individuals (7). However small amounts of fibrinogen split products are found even in serum from healthy individuals, and it is hard to conceive that the coagulation and fibrinolytic systems virtually exhibit zero activity even in the "normal" state. The persons studied here presented no clearcut coagulation abnormality apart from a moderately elevated fibrinogen level (Table I), and would thus not fulfil the *usual* criteria of intravascular continuous coagulation. However the changes in the At III level suggest the presence of a low grade intravascular coagulation, which was suppressed during anticoagulant therapy. Since all the individuals studied probably had some degree of arteriosclerotic disease, we do not know whether these results have a bearing on the condition in normal individuals.

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Acta Med. scand. Vol. 193 pp. 311–318 1973

THE STUDY OF WOMEN IN GOTHENBURG 1968–1969 —A POPULATION STUDY

General Design Purpose and Sampling Results

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Abstract. A population study of women in Gothenburg, Sweden, is presented. The women were representative of the total female population in Gothenburg in the age strata 38, 46, 50, 54 and 60 years. Altogether 1 462 women participated in the examination, the participation rate being 90.1%. Social data of participants and non-participants are given. The performance of the examination is described and research projects are outlined. It is concluded that the selection of a strictly representative sample, together with careful uniformity of performance and high participation rate, will make it possible to draw valid conclusions about the total population of middle-aged women in Gothenburg.

Population studies around the world have proved to be a useful complement to clinical studies. In Sweden many of the essential requirements for population studies are fulfilled. From the up-to-date Revenue Office Register a representative population sample can easily be obtained. There is also good collaboration between general practitioners and hospital physicians. Health surveys are often requested by Swedish people today which ensures a high participation rate. Earlier population studies in Gothenburg on women (8) and on men (16) have given valuable experiences.

The present population study on women was started in Gothenburg in 1968. The previous study of women in Gothenburg related primarily to the prevalence of iron deficiency and its causes and showed also that certain changes in plasma lipids occurred during the climacteric (9). Previous studies on coronary heart disease (CHD) in women have indicated that the incidence of CHD increases after the menopause. In the previous study of women the age groups were not sufficiently large to allow correlative studies between various variables or to form a basis for a prospective study. In the present study five age groups were studied, with a greater number of individuals in each age stratum. Emphasis was placed on the ages around the menopause.

The purpose of the present paper is to outline The Study of Women in Gothenburg 1968–69: design, main purposes, participation rate, characteristics of participants and non-participants.

PRESENTATION OF THE INVESTIGATION

Procedure

A random sample of women in five age strata (ages 38, 46, 50, 54 and 60) was studied. The sample was obtained from the Revenue Office Register and consisted of women born on certain dates as shown in Table I, and who at the beginning of 1968 were living in Gothenburg (population 445 000). Further details of the population of Gothenburg have been given previously (16). The way in which the sample was selected ensured that it was representative of the total female population in Gothenburg in these ages as of Jan. 1968.

An invitation was sent to the women, including a description of the investigation, offering them a free health examination. It was pointed out that their participation was of great importance for the investigation as a whole. Those born at the beginning of the year were called first. The survey was performed for the most part during a 12-month period. In this way the influence of age differences within each age group was reduced as much as possible (Table II). The rates obtained for pathological conditions in the population may thus

Table I. *Principles for selection of the material*

Year of birth	Date of birth				
1930	6	12	18	24	30
1922	6	12	18	24	30*
1918	6	12	18	24	30*
1914	6	12			
1908	6				

Of the women born on the 30th only those born in Jan.–June were called for the examination.

Table II. *Age at the time of the primary examination*

Year of birth		Mean age (y.)	S D
1930	372	38.59	0.22
1922	431	46.57	0.21
1918	398	50.55	0.20
1914	180	54.56	0.24
1908	81	60.87	0.24

be said to represent point prevalence rates, assessed on a rolling census day. For menstruating women an examination date between the 10th and 20th day of the menstrual cycle was chosen if possible.

After a pilot study of medical students, the examination of the population sample started in May 1968. During May and June 1968 a study of 54 women was made. Only very slight modifications in the procedure were introduced before the main examination started in Sept. 1968. Consequently for most of the parameters, the results from May and June 1968 could be used together with the others. Modifications will be pointed out when the results from the various examinations are presented separately.

Up to June 1969 1 435 women had been examined, corresponding to 98.2% of all the participants examined. In the autumn of 1969 another 27 women (1.8% of the participants) were examined until the primary examination was completed in Jan. 1970.

When the time for the examination had been settled, two questionnaires were sent out. One contained gynecological questions, the other general questions about, for example, previous illnesses and hospitalizations and diseases occurring among their nearest relatives. The women were asked to come after an overnight fast but were allowed water in the morning.

The examination took place at Sahlgren's Hospital in Gothenburg. The research staff was the same during the whole examination period.

Performance of the primary examination

The women were called at intervals of a quarter of an hour between 7.00 and 9.45 a.m. They passed the examination stations in the same order as shown in Table III, except those 38 years of age, who followed a somewhat modified program.

At the first station a nurse checked the women's questionnaires and helped them to complete them if necessary. The time for the last voiding of urine was noted. If they had drunk water in the morning, the amount was estimated and registered. Urine specimens were voided under sterile precautions into sterile paper cups and transmitted to sterile glass tubes which were then immediately put in the refrigerator (+4°C).

At the second station a general physical examination was performed. The arterial BP was measured in the sitting, lying and standing positions. A mercury manometer with a 12 cm cuff was used. The eyegrounds were examined without dilating the pupils. The information given in the questionnaires was supplemented by further questions about symptoms and family history of heart diseases, hypertension, kidney diseases, diabetes, and diseases of the joints. The WHO standard questionnaires for angina pectoris, for intermittent claudication and for chronic bronchitis were used. The women were also asked about smoking and drinking habits.

A blood sample was then taken at the third station with the participant supine. Approximately 120 ml blood was drawn from a cubital vein by means of a disposable steel needle (i.d. 2.0 mm). The tourniquet was released as soon as the vein was entered. About 60 cm of plastic tubing was attached to the needle. The blood was collected in two 100 ml centrifuge glass tubes. The blood collection could thus take place out of sight of the participant. Five ml blood was mixed with 6 mg EDTA and used for routine hematological investigations. Another 1.6 ml blood was collected in a syringe containing 0.4 ml 3.8% sodium citrate solution. This specimen was used to determine the ESR by the Westergren method. The remaining blood was left to clot at room temperature for 2–3 hours. After clotting, the blood was centrifuged and the serum separated. Serum which could not be analysed fresh was stored at −20 or −60°C in plastic cups. Some serum was freeze-dried and kept at +4°C under nitrogen gas in glass ampoules. Approx-

Table III. *Order of stations at the primary examination (time at each station 15 min)*

Women 46, 50, 54 and 60 y	Women 38 y
1 Urine sampling	1 Urine sampling
2 Examination and interview by internist	2 Examination and interview by internist
3 Blood sampling	3 Blood sampling
4 Examination of the teeth	4 Examination and interview by hematologist, 1st time
5 Examination and interview by hematologist	5 Whole body counter examination, 1st time
6 ECG	6 ECG
7 Gynecological examination and interview	7 Gynecological examination and interview
8 Interview by dietician	8 Interview by dietician
	9 Examination of the teeth
	10 Examination and interview by hematologist, 2nd time
	11 Whole body counter examination, 2nd time

Table IV *Examinations following the primary examination for different subgroups*

Iron abs. = iron absorption study, Work = work performance test, Breast = breast examination, Psych. = psychiatric examination, Body c. = body composition study, IVGTT = i.v. glucose tolerance test with insulin response, OGTT = oral glucose tolerance test and microangiopathy studies

Examination	Age (y.)	Date of birth 6	12	18	24	30
Second	38	Iron abs.	Iron abs.	Iron abs.	Iron abs.	Iron abs.
	46	Work[a] Breast[b]	Psych.	Psych.	Psych.	Psych.
	50	Work[a] Breast	Psych.	Psych.	Psych.	Psych.
	54	Work[a] Breast[b]	Psych.			
	60	Work[a] Body c.				
Third	38	Work Breast[b]	Body c.	Psych.	Psych.	—
	46	—	Body c.	—	—	—
	50	IVGTT	IVGTT	IVGTT	IVGTT	IVGTT
	54	—	Body c.			
	60	Breast				
Fourth	38	—	—	—	—	—
	46	—	—	—	—	—
	50	OGTT[a]	Body c.	—	—	—
	54	—	—			
	60	—				

[a] Born in an odd month. [b] Born in an even month.

imately 20 ml blood was drawn in heparin tubes and centrifuged. The plasma was stored in plastic cups at −20°C.

At the fourth station X-ray of the teeth (orthopantomogram) was performed and colour slides of the teeth and gums were taken. Questions about oral hygiene habits, home care and visits to dentist were asked.

At the fifth station the women were asked about symptoms of anemia, previous iron therapy, infections during the last month, with special reference to infection as causes of anemia or high ESR, and history of allergic manifestations. Anthropometric measurements were also made, including length, weight, circumference of the upper arm, waist and hips, subscapular and triceps skinfold, and epicondylar width at the knees and wrists.

At station 6 a standard 12 lead ECG was taken. The participants were then offered coffee or tea and sandwiches. During this pause they answered a psychiatric questionnaire consisting of Eysenck Personality Inventory, a phobia check list, and a hypochondriasis inventory.

At the seventh station the gynecological questionnaire was completed, and gynecological examination including Papanicolaou smear was performed. Most postmenopausal women were asked to make a 24-hour collection of urine in specially designed plastic containers for determination of estrogen excretion. All 38-year-old women and subsamples of women aged 46 and 50 were given sanitary towels and tampons and instructed to collect material for determination of menstrual blood loss.

At the eighth station the women were interviewed by a dietician about their dietary habits. In all subjects a 24-hour recall was made, and on a subgroup constituting about one fourth of the total sample a dietary history according to a questionnaire was taken as well.

Extensive hematological studies were carried out on the 38-year-old women and they therefore followed a modified program. The ^{59}Fe absorption was measured using a whole body counter, and bone marrow smears were prepared on aspirated material from the sternum on those who agreed to this examination.

Subsequent examinations

Different studies were made in subgroups of the sample depending upon their date of birth. The subdivision of the material for these examinations is summarized in Table IV.

Outline of research projects

A great number of research projects based on this material will be described in subsequent reports. The present paper gives a broad outline of some of the purposes of the projects mainly to illustrate the design of the study. Examples of problems to be studied are given in Table V.

Hematology. In the 38-year-old women a special study was made on the prevalence and symptomatology of iron deficiency and the reliability of parameters for this diagnosis. A double-blind study with iron and placebo was also made in these women, at which time the subjective change in wellbeing was noted and the change in psychometric tests recorded. In this way the iron deficiency state could be studied from several points of view in the same subjects.

Blood coagulation, fibrinolysis and platelet function. Studies on blood coagulation, fibrinolysis and platelet function were performed on subsamples of each age group. The purpose was to outline the age distribution of these variables and to correlate them with other data

Table V. Research projects

Project	Problems to be studied (examples)
Hematological studies	1. "Normal" hematological values 2. Prevalence of anemia and iron deficiency 3. Causes of anemia 4. Symptomatology of anemia and iron deficiency 5. Reliability of parameters for the diagnosis of iron deficiency
Cardiovascular studies	1. Prevalence of rheumatic heart disease and CHD 2. Characteristics of women with heart disease 3. Prevalence of risk factors for CHD 4. BP—normal values 5. Prevalence of arterial hypertension 6. Characteristics of women with arterial hypertension
Work performance studies	1. Working capacity in women 2. Prevalence of CHD as diagnosed by means of work performance test
Studies on renal function and urinary tract diseases	1. "Normal" values 2. Prevalence of kidney diseases and urinary tract infections 3. Causes of kidney diseases and urinary tract infections 4. Characteristics of women with kidney diseases and urinary tract infections
Gynecological studies	1. Prevalence of various gynecological diseases including precancerous states of the cervix 2. Prevalence of symptoms traditionally related to the menopause 3. Excretion of estrogen and estrogen metabolites in the urine of postmenopausal women
Psychiatric studies	1. Prevalence and incidence of mental disorders 2. Analysis of causative factors 3. Analysis of psychosomatic interrelationships
Studies on carbohydrate metabolism	1. Normal values 2. Prevalence of clinical and asymptomatic diabetes mellitus 3. Prevalence of low early insulin response after i.v. glucose infusion 4. Characteristics of women with low early insulin response
Coagulation studies	1. Normal values 2. Prevalence of coagulation disorders
Body composition studies	1. Normal values 2. Data of body composition studies in relation to anthropometric data 3. Data of body composition studies in relation to food intake
Dietary studies	1. Dietary habits in the population 2. Dietary habits in relation to obesity, prevalence of CHD, glucose tolerance, dental status, etc.
Breast examination	1. Prevalence of breast tumours 2. Reliability of various examination methods for the diagnosis of breast tumours
Dental studies	1. Prevalence of various dental diseases 2. Oral hygiene habits in relation to dental state

such as anthropometric measurements, serum lipids and data on smoking habits.

Coronary heart disease. Characteristics including risk factors of women with different manifestations of CHD participating in the population study and of women with myocardial infarction living in the same area (17) were studied. A comparison was made with the total population sample.

Blood pressure. Women with systolic BP ≥ 160 mmHg and a diastolic BP ≥ 96 mmHg, and who were not on antihypertensive treatment, were offered further controls and were submitted to a controlled trial on antihypertensive drugs.

Work performance test. Subgroups were admitted to a maximal work performance test on an electronically braked bicycle ergometer. The working capacity and the frequency of abnormalities of the ECG in the population were studied.

Bacteriuria. Women with socalled significant bacteriuria were treated according to certain schedules and submitted to follow-up studies. A urine concentration test was performed on all individuals (13 hours of thirst). Those concentrating to less than 600 mOsm/kg H_2O were examined further with the pitressin tannate test.

Gynecology. All women were interviewed about symptoms and especially about those considered to be related to the menopause. The postmenopausal women were compared with the 38-year-old women. In this way a control group was obtained, and the study included a search for differences between the postmenopausal women and the control group.

Psychiatry. Excluding the pilot study a subsample of 800 women underwent a thorough psychiatric interview including detailed history, behaviour observation and questionnaire response. The goal was to assess the prevalence and incidence of psychiatric disorders in the population and to relate these morbidity parameters to other biosocial variables.

Carbohydrate metabolism. Excluding the pilot study all 50-year-old women were asked to participate in a subsequent study including an i.v. glucose tolerance test with estimation of the early insulin response. Those with glucose intolerance and/or low insulin response were submitted to further examinations. A subsample of the 40-year-old women acted as a control group, and was also studied further.

Body composition studies. Studies using isotope dilution techniques were performed on a subsample in order

to estimate body cell mass, extracellular water and body fat. In this way the anthropometric measurements could be compared with the body composition data.

Breast examination. The breasts of all women were examined by the gynecologist in connection with the gynecological examination. In addition a subgroup of women was asked to come back for a further examination of the breasts including palpation of the breasts by a specially trained surgeon, an X-ray examination of the breasts and infrared thermography.

Odontology. The dental status was noted and the prevalence of dental diseases recorded. Special interest was directed to paradontitis.

Follow-up

Women with symptoms or signs of diseases were offered further control by members of the research staff or referred to specialists or general practitioners.

Computer processing

All data obtained in the various examinations were converted into punched cards by means of an optical mark page reader and then processed by a computer (IBM 360 65). Altogether the data comprised more than 1 500 variables (about 50 punched cards) for each participant (breast examination, dental examination and dietary interview not included).

RESULTS OF SAMPLING

Response to examination

As seen in Table VI, 1 622 women were selected initially for the examination, of whom 1 462 attended the examination, thus giving an overall participation rate of 90.1%. The participation rate was lowest in the oldest age groups. Some women had died or moved from the city between the date of sampling at the Revenue Office and the intended examination date. If these women are excluded, the attendance rate among the remaining 1 594 was 91.7%.

The refusers were as a rule willing to give information about themselves. In this way detailed information was obtained through interviews from

Table VII. *Non-participants*

Age (y.)	Dead	Moved from Gothenburg	Inaccessible	Refused	Total
38	3	7	1	24	35
46	0	8	2	38	48
50	3	4	0	31	38
54	2	0	1	20	23
60	0	1	0	15	16
Total	8	20	4	128	160

Table VI. *Participation rate in different age groups*

Age (y)	Called for examination (*n*)	Came for examination (*n*)	Participation rate ( )
38	407	372	91.4
46	479	431	90.1
50	436	398	91.0
54	203	180	88.6
60	97	81	83.5
Total	1 622	1 462	90.1

another 7.3% of the total material, i.e. from most of the non-participants. Examination at their homes was not performed, as previous experience indicated that very few relevant data could be expected (16).

Some social data of participants and non-participants

There were 160 non-participants, as seen in Table VII. Of these, eight had died during the interval between the sampling and the intended examination date. Another 20 women had moved from Gothenburg during the year. Only four women could not be contacted.

Data about place of birth and marital status of the women who refused to participate in the examination are given in Tables VIII and IX and compared with those of the participants. These data were obtained from the Revenue Office Register. There was no difference between participants and non-participants concerning place of birth, while there is a preponderance of unmarried and divorced women and widows among the non-participating women (χ^2-test, $p < 0.001$).

DISCUSSION

Reasons for using an epidemiological approach

A total population is characterized. The advantages of studying a sample representative of a total population are obvious. In this way knowledge about the "normal" state of a population is obtained, and multiple causality of diseases may be illuminated, since the subjects are studied simultaneously from several points of view. Subjects with various diseases may be compared with those of the total sample. Thus there is no lack

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Project	Problems to be studied (examples)
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Table VIII *Place of birth (%) of participants and non-participants*

Age (y.)	City of Gothenburg and surroundings	Other parts of Sweden	Other parts of Scandinavia	Other parts of Europe	Outside Europe
Participants (n = 1462)					
38	57.3	28.2	7.5	5.9	1.1
46	50.1	40.8	5.6	3.5	—
50	50.0	45.0	3.8	1.3	—
54	57.8	37.8	3.3	0.6	0.6
60	59.3	38.3	1.2	1.2	—
Total	53.4	38.2	5.1	3.0	0.3
Refused (= 128)					
Total	60.7	33.9	4.7	0.8	—
All non-participants (= 160)					
Total	55.1	35.2	5.7	3.1	0.6

of controls, which is otherwise often a major problem.

Population studies give reference material for case control studies. Lack of suitable control material is, as a rule, the great difficulty in evaluating the difference in signs, symptoms and laboratory data between patients with any given disease and "normal" people (13). The previous population study performed on men in Gothenburg (16) has proved to be of great importance for such case control studies, as for example comparison of men with myocardial infarction with those of the total population (17). In the same way this study is meant to provide reference material for case control studies in women.

Table IX. *Marital status ( ) of participants and non-participants*

Age (y.)	Unmarried	Married	Divorced or separated	Widows
Participants (1462)				
38	7.5	81.7	9.2	1.6
46	8.1	79.1	10.2	2.6
50	7.5	77.9	9.8	4.8
54	10.6	72.8	8.9	7.8
60	12.3	65.4	8.6	13.6
Total	8.3	77.9	9.6	4.2
Refused (= 128)				
Total	24.5	54.3	13.4	7.9
All non-participants (n = 160)				
Total	22.8	54.3	15.2	7.5

Why study women?

Most population studies have dealt with men. The main reason for this is probably that CHD which has been shown to be especially suitable for study by an epidemiological technique, is more frequent in men. This makes population studies of men more remunerative.

Studies of men and women from the same population may explain reasons for sex differences in diseases. In Gothenburg a population study of 50-year-old men was performed in 1963 (16). The present study utilized the same methods as in that study The men were reexamined at the age of 54 in 1967 Thus, in the present study the data of the women aged 50 and 54 may be compared with those of the men obtained in 1963 and 1967

If for any reason, a disease is more common in one sex than in the other the reason may be obtained by studying the cause of the higher incidence in the one sex or by trying to find out the cause of the lower incidence in the other The latter approach may be especially important if the sex differences are not the same for all periods of life. As an example, CHD is much more common in men than in women at younger ages, but after the menopause the difference is less.

Population studies on women have, as a rule, been restricted to some special diseases, such as carcinoma of the cervix and the breast or iron deficiency However fatigue may be due to an endogenous depression, and anemia may be explained by heavy menstrual blood loss. Thus it is of great importance to illuminate the sample simultaneously from several aspects. The present sample of women has probably been studied from a larger number of viewpoints than any extensive previous population material.

Methodological aspects

Age strata in relation to age intervals. Age strata have been used in order to eliminate the effect of age differences within the age groups. This will improve the possibility of studying factors of importance other than age within the groups. The reduced intragroup age variation facilitates

Table X. *Participation rate in the present and some earlier population studies*

R = random sample of population, T = total population

Place	Type of sampling	Sex	Age group	No. of participants	Participation rate (% of selected samples)
Busselton, Australia (2)	T	♂+♀	>21	3 403	91
Dalby Sweden (5)	T	♂+♀	All	2 520	98.8
Dalby Sweden (7)	T	♂+♀	All	3 261	93.5
Evans County USA (12)	R	♂+♀	15–39	3 102	92
	T	♂+♀	40–74		
Framingham, USA (3)	R	♂+♀	30–59	4 469	68.6
Glostrup, Denmark (6)	R	♂	50	436	85
		♀	50	366	79
Gothenburg, Sweden (16)	R	♂	50	855	88
Gothenburg, Sweden (8)	R	♀	15–50	663	88.6
Gothenburg, Sweden	*R*	♀	*38–60*	*1 462*	*90.1*
Ilomantsi and Poytyä, Finland (1)	R	♂+♀	>20	906	82.3
Manhattan, USA (11 15)	R	♂+♀	20–59	1 660	87
Rhondda Fawr Wales, UK (4)	T	♀	>20	939	93.4
Tecumseh, USA (14)	T	♂+♀	All	8 621	88.0
Tecumseh, sample V USA (14)	T	♂	All	416	90.6
	T	♀	All	430	91.7
	T	♂+♀	30–39	137	97.2
	T	♂+♀	40–49	95	96.0
	T	♂+♀	50–59	54	78.3
	T	♂+♀	60–69	35	68.4
Wensleydale, UK (10)	T	♂	>15	182	69
		♀	>15	230	82

The present study

studies on the effect of age in the comparison of different groups. It may thus be expected that smaller real changes with age can be detected with higher statistical significance, and smaller groups can be used.

Uniformity in performance. In order to reduce, as far as possible, the variability in results due to variations in examination methods great efforts were made to standardize the procedures. Thus all participants came fasting for the primary examination, which was always performed in the morning. As seen in Table III the women passed the different examination stations in the same order. As examples of how to avoid interobserver variation, one and the same doctor measured all the BPs, another made all the anthropometric measurements, and the subsample of women submitted to the psychiatric examination was interviewed by one and the same psychiatrist. Some remaining sources of systematic variation, such as the effect of seasonal variation, will be studied separately. The strict uniformity of performance has been considered to be of great importance in the study of the differences between subgroups of our material and when comparing our results with those of other population studies.

Comments on the participation rate. Table X gives the participation rate for some earlier population studies. It is seen that a higher participation rate is to be expected in younger people. In this investigation more than 90% of the total sample came for the examination. Detailed information was obtained through interviews from most of the non-participants. Further information on the non-participants was obtained by studying the records of inpatient and outpatient clinics, insurance and social registers. There was also a high participation rate in the various subgroup examinations. The high response to the investigation, together with all the information obtained from the non-participants, will make valid conclusions about the total female population in these ages possible.

Evaluation of screening methods. There is a great interest in health screening all over the world. However the knowledge is often insufficient when health or sickness is to be determined according to a certain symptom or group of symptoms or certain laboratory values. By combining screening methods with approved and usually more time-consuming examinations, a further knowledge about the value of the screen-

ing methods will be obtained. The measurement of height and weight and other anthropometric data are examples of this in the present study These data have been correlated to those of body compartments, calculated from isotope dilution measurements in order to find out whether they serve as an acceptable screening method for obesity and, if so, which of the anthropometric data correlate best with the data from the body composition studies.

Future studies may give information on the correlation between various symptoms and signs and laboratory data, on the one hand, and the development of illness on the other For example, BP may be correlated to the development of stroke. In that way information can be obtained on what is to be considered "pathological" "boundary or "normal"

ACKNOWLEDGEMENTS

This investigation was supported by grants from R. and V Andréns fond, Anna Linds donation, AB Astra, Forenade Liv Forskningsfonden id Konung Gustaf V:s Jubileumsklinik, Goteborg, Stiftelsen S enska Naringsforskning, Svenska Margarinindustrins Förening för Naringsfysiologisk Forskning and Svenska Nationalforeningen mot hjart- och kärlsjukdomar

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Acta med. scand. Vol. 193 pp. 319–323 1973

THE DIAGNOSIS OF PULMONARY EMBOLISM WITH GAMMACAMERA

A Comparison with Clinical, Radiological and Electrocardiographic Findings

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Abstract. A retrospective analysis has been performed on patients investigated with pulmonary scintigraphy on suspicion of pulmonary embolism. Seventy-four patients have been investigated during a 2-year period and a comparison has been made between clinical diagnosis, scintigraphy, chest X-ray and ECG. The method of scintigraphy is described and evaluated. Scintigraphy is of considerable value in the diagnosis of pulmonary embolism as it is a simple procedure for the patient, well correlated to clinical findings, as well as giving good quantitative data. It also allows for an evaluation of therapeutic results. Chest X-rays provide poor information, and characteristic ECG changes are only seen in the most advanced cases.

The purpose of the present study was to evaluate different methods of investigation including scintigraphy, radiology and ECG as regards their validity in relation to pulmonary embolism as diagnosed clinically. Several previous investigations have pointed to a high incidence as well as to the diagnostic difficulties associated with this condition (4, 5, 8).

METHODS

Pulmonary scintigraphy with gammacamera has for the last two years been used as a diagnostic tool at Danderyd Hospital. Radioactive particles, somewhat larger than the internal diameter of pulmonary capillaries, are injected intravenously giving rise to pulmonary microembolism. The γ-radiation emitted from these particles is sensed by the camera, which registers the capillary perfusion in the form of a picture.

In the present investigation 88 scintigrams have been evaluated. The first 20 involved the use of macroaggregated albumin (^{131}I-MAA, Amersham, England). The mean size of these particles was 29 μm and the administered mean activity 300 μCi. Thereafter we used ^{113m}In(OH)$_3$-MAA (10) with a mean particle size of 32 μm in 32 instances, involving a mean activity of 2 mCi. In the last 36 examinations we used ^{99m}Tc-sulphur colloid-MAA (^{99m}T S-MAA), which is a modification of that used by Cragin et al. (1). The technique of preparation has recently been modified and standardized. The presently used injectate has a radioactive concentration of 2–10 mCi/ml and an albumin content of 0.5 mg/ml. The number of particles is about 2–3 × 10^6/ml and the biological half-life in mice experiments about 5 hours. The maximum injected was usually 1 ml, i.e. 0.5 mg albumin corresponding to 2–3 × 10^6 particles with an average diameter of 15–20 μm (Fig. 1).

All patients were investigated lying horizontally, usually from the anterior, lateral and posterior side. In a few severely ill patients only anterior and lateral registrations were possible. The first investigation was performed as early as possible after onset of symptoms. In a few cases with positive findings the investigation was repeated 1–4 days and a further week later.

The interval between scintigraphy and the chest X-ray on the one hand, and the ECG on the other did not usually exceed 24 hours. The maximum interval was 2 days.

Scintigraphic criteria

Segmental defects on the scintigram have usually been interpreted as being caused by emboli. The scintigram was always compared with simultaneous chest X-rays in order to exclude other possible causes for these defects. A prerequisite was that these defects follow a vascular distribution. In grading the extent of the embolic process a rough estimate of the non perfused pulmonary tissue was made as follows (Fig. 2).

Grade I part of a pulmonary segment.
Grade II complete pulmonary segment.
Grade III more than one pulmonary segment.

Radiological criteria

The X-rays were scrutinized with special reference to the presence of segmentally shaped parenchymatous infiltrates, pleural effusions and raised diaphragms, as well as signs of circulatory obstruction (3).

ing methods will be obtained. The measurement of height and weight and other anthropometric data are examples of this in the present study. These data have been correlated to those of body compartments, calculated from isotope dilution measurements in order to find out whether they serve as an acceptable screening method for obesity and, if so, which of the anthropometric data correlate best with the data from the body composition studies.

Future studies may give information on the correlation between various symptoms and signs and laboratory data, on the one hand, and the development of illness on the other. For example, BP may be correlated to the development of stroke. In that way information can be obtained on what is to be considered "pathological" boundary or "normal".

ACKNOWLEDGEMENTS

This investigation was supported by grants from R. and V. Andréens fond, Anna Linds donation, AB Astra, Forenade Li Forskningsfonden, od Konung Gustaf V:s Jubileumsklinik, Göteborg, Stiftelsen Svensk Näringsforskning, Svenska Margarinindustrins Förening för Näringsfysiologisk Forskning and Svenska Nationalföreningen mot hjärt- och kärlsjukdomar.

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Acta med. scand. Vol. 193 pp. 319–323 1973

THE DIAGNOSIS OF PULMONARY EMBOLISM WITH GAMMACAMERA

A Comparison with Clinical Radiological and Electrocardiographic Findings

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Abstract. A retrospectiv analysis has been performed on patients investigated with pulmonary scintigraphy on suspicion of pulmonary embolism. Seventy-four patients have been investigated during 2-year period and comparison has been made between clinical diagnosis, scintigraphy chest X-ray and ECG. The method of scintigraphy is described and evaluated. Scintigraphy is of considerable alue in the diagnosis of pulmonary embolism as it is simple procedure for the patient, well correlated to clinical findings, as well as giving good quantitative data. It also allows for an evaluation of therapeutic results. Chest X-rays provide poor information, and characteristic ECG changes are only seen in the most advanced cases.

The purpose of the present study was to evaluate different methods of investigation including scintigraphy radiology and ECG as regards their validity in relation to pulmonary embolism as diagnosed clinically Several previous investigations ha e pointed to a high incidence as well as to the diagnostic difficulties associated with this condition (4, 5 8).

METHODS

Pulmonary scintigraphy with gammacamera has for the last two years been used as diagnostic tool at Danderyd Hospital. Radioactive particles, somewhat larger than the internal diameter of pulmonary capillaries, are injected intravenously giving rise to pulmonary microembolism. The γ-radiation emitted from these particles is sensed by the camera, hich registers the capillary perfusion in the form of picture.

In the present investigation 88 scintigrams have been evaluated. The first 20 involved the use of macroaggregated albumin (^{131}I-MAA, Amersham, England). The mean size of these particles as 29 μm and the administered mean activity 300 μCi. Thereafter we used ^{113m}In(OH)$_x$-MAA (10) with mean particle size of 32 μm in 32 instances, involving mean activity of 2 mCi. In the last 36 examinations we used ^{99m}T -sulphur colloid-MAA (^{99m}T S-MAA), hich is modification of that used by Cragin et al (1). The technique of preparation has recently been modified and standardized. The presently used injectate has radioactive concentration of 2 10 mCi/ml and an albumin content of 0.5 mg ml. The number of particles is about 2 3 10^{6} ml and the biological half-life in mice experiments about 5 hours. The maximum injected was usually 1 ml, i e. 0.5 mg albumin corresponding to 2 3 10^{6} particles with an average diameter of 15–20 μm (Fig. 1).

All patients were investigated lying horizontally usually from the anterior lateral and posterior side In few severely ill patients only anterior and lateral registrations were possible. The first investigation was performed as early as possible after onset of symptoms. I few cases with positive findings the investigation was repeated 3–4 days and further week later

The interval between scintigraphy and the chest X-ray on the one hand, and the ECG on the other did not usually exceed 24 hours. The maximum interval was 2 days.

Scintigraphic criteria

Segmental defects on the scintigram have usually been interpreted as being caused by emboli. The scintigram was always compared with simultaneous chest X-rays in order to exclude other possible causes for these defects. A prerequisite was that these defects follow vascular distribution. In grading the extent of the embolic process ough estimate of the non-perfused pulmonary tissue was made as follows (Fig. 2).

Grade I part of pulmonary segment.
Grade II complete pulmonary segment.
Grade III more than one pulmonary segment.

Radiological criteria

The X-rays were scrutinized with special reference to the presence of segmentally shaped parenchymatous infiltrates, pleural effusions and raised diaphragms, as ell as signs of circulatory obstruction (3).

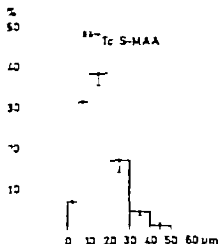


Fig. 1. Size distribution of ^{99m}Tc S-MAA particles. The bars indicate S.E.M. for 8 preparations.

ECG criteria

The ECG criteria for acute right ventricular strain were:

1. S-T and/or T lowering either of recent date or increasing in leads CR_1 and CR_4 close to onset of symptoms or decrease of these changes during the following days.

2. Addition of complete or incomplete RBBB.

PATIENTS

There were 74 patients aged 29–88 (mean 60) years, one of whom had been investigated on two different occasions. Patients who were subjected to scintigraphy because of suspected acute pulmonary embolism and investigated within one week of onset of symptoms, or were still symptomatic, have been included together with one group of patients without pulmonary symptoms but with objective signs of deep vein thrombosis. The patients were grouped as follows.

Group A. Thirty-four patients with symptoms and physical findings which, with a high degree of probability were due to pulmonary embolism. The symptoms included were sudden onsets of dyspnoea, chest pain, collapse, circulatory arrest, cough, haemoptysis and nausea. The physical findings included were hypotension, tachycardia, cyanosis, rales or crepitations. Cases with other known causes of these symptoms were excluded.

Group B. Thirty-two patients with similar symptomatology and signs as above, but of a less severe degree, and where less certainty was felt as regards the diagnosis.

Group C. Eight patients, with only a tentative diagnosis of pulmonary embolism. The symptoms were subsequently shown to be due to acute myocardial infarction, heart failure and/or pneumonia.

Group D. Eleven patients with deep vein thrombosis diagnosed by plethysmography and/or phlebography but without clinical symptoms of pulmonary embolism.

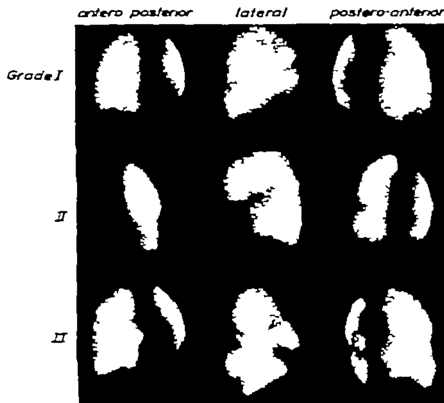


Fig. 2. Grades of perfusion defects in the scintigrams.

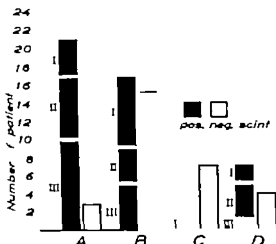


Fig. 3. Clinical groups A–D and scintigraphic grades (I–III) of the perfusion defects in the scintigrams.

RESULTS

Out of the 75 scintigrams performed positive findings were seen in 46. These were of grade I severity in 15 grade II in 15 and grade III in 16 patients.

In group A (24 patients) 21 had positive scintigrams (Fig. 3) grade I in 4 grade II in 7 and grade III in 10 patients.

In group B (32 patients) positive scintigrams were seen in 17 In 8 patients these were of grade I, in 4 of grade II and in 5 of grade III.

In group C only 1 of the 8 patients had positive findings, which were of grade I severity

In group D (11 patients) 7 were found with positi e findings, which in 2 were of grade I, in 4 grade II and in 1 patient of grade III severity

A comparison between scintigraphic and radiological findings was also performed (Table I). There was poor agreement, although the radiological criteria were fairly generous. Thus no radiological findings were seen in 30 out of the 46 patients with an abnormal scintigram.

Table I shows also a comparison between the results of the scintigram and ECG findings. Ten of 12 patients with positi e ECG findings also had grade III changes on the scintigram, whereas the remaining 2 scintigrams were considered normal. Of these 12 patients 3 had changes according to both criteria, whereas the remaining 9 fulfilled only the first.

Taking all three methods of in estigation together, most positive findings were encountered in the patients belonging to group A (Fig. 4). The scintigram was positive in 1 of 4 the X-ray in 13 of 23 and the ECG in 9 of .3 patients.

A lower incidence of positive findings with these methods is seen in group B (Fig. 5). The scintigram was positi e in 18 of 32 patients, the chest X ray in 7 of 31 and the ECG in 4 of the 32 patients. Also in this group the scintigram was best found to reflect the clinical picture. No deaths occurred among the patients.

Table I. *Comparison between scintigraphic radiological and ECG findings*

	Scintigraphy		
	Pos.	Neg.	Σ
X-ray			
Pos.	16	9	25
Neg.	30	17	47
Σ	46	26	
ECG			
Pos.	10	2	12
Neg.	35	22	57
Σ	45	.4	

DISCUSSION

Pulmonary angiography is considered as the most accurate method available in the diagnosis of

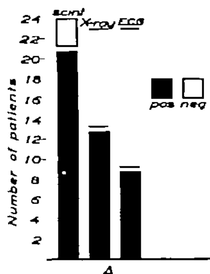


Fig. 4. Comparison between scintigraphic, and ECG findings in group A.

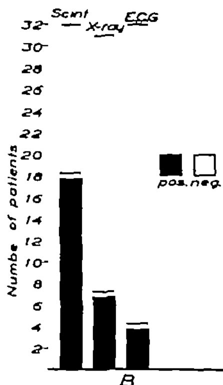


Fig. 5. Comparison between scintigraphic, radiological and ECG findings in group B.

pulmonary embolism. However Moser et al. (6) showed that typical angiographic findings are not always obtained in experimentally induced embolism. Furthermore pulmonary angiography must be regarded as a very advanced procedure in these often critically ill patients, as well as being time-consuming and requiring considerable resources in staff. Right-sided catheterization is necessary and large amounts of contrast medium have to be injected. These manipulations are not particularly suited to patients with thrombo-embolic disease. Pulmonary scintigraphy on the other hand is simple and can easily be repeated within short intervals, allowing useful evaluation of therapy. Furthermore, in several clinical investigations (7), agreement between the scintigrams and angiography has been found in about 75% of cases.

The segmental defects observed on the scintigram are, of course, not absolute proof of pulmonary embolism. Similar defects may be caused by any process which affects the pulmonary vasculature, i.e. atelectases, localized tumours. It is therefore essential to evaluate scintigrams in conjunction with simultaneously obtained chest X rays.

Chest X-rays seem a poor aid in the diagnosis of pulmonary emboli. Emboli which have given rise to extensive changes on the scintigram (grade III) were not associated with a higher incidence of positive radiological findings than emboli with scintigrams of grade I changes.

The ECG shows characteristic changes almost only in cases which have very advanced degrees of pulmonary embolism on the scintigram. The ECG seems of little diagnostic value in patients who have borderline symptomatology or when the scintigram shows moderate changes. The sensitivity of ECG is thus low which is illustrated by the fact that, even in the patients of group A 14 of 23 had non-conclusive ECG findings. A review of these ECGs does not suggest that any alteration in criteria will improve the sensitivity of ECG in pulmonary embolism. It should be stressed, however in view of the well known transient nature of the ECG changes, that ECG in some cases was performed up to 7 days after onset of symptoms. Five of those 7 patients in groups A and B who had large scintigraphic changes (grade III) and in whom the ECG was recorded during the first 24 hours, thus had positive ECG findings. Of the patients in the same groups in whom ECG was recorded within 48 hours, the positive ECGs numbered 6 out of 10.

The incidence of positive ECG findings in this study is considerably lower than in the series described by Dunér et al. (2) who employed the same ECG criteria. An explanation of this discrepancy is that the patients in the study of Dunér et al. were considered to have pulmonary embolism based on clinical findings, i.e. roughly corresponding to those in group A, and the ECGs were taken earlier after onset of symptoms.

Considering the high rate of emboli in the patients belonging to group D who were known to have deep vein thrombosis but who did not have any pulmonary symptoms, it would seem that pulmonary emboli without giving any symptoms may be fairly common. This is consistent with the common autopsy finding of smaller pulmonary emboli not associated with any symptoms.

Lung scintigrams involve microembolism of small albumin particles in the pulmonary capillaries. Weibel (9) has estimated the total number of alveolar capillary segments in the human lung

at 28×10^{10}. An injection of $2\text{–}3 \times 10^{6}$ albumin particles could therefore theoretically block one capillary in 100 000. The risk of affecting pulmonary function is therefore negligible.

In this procedure we no longer use ^{131}I MAA, as it subjects the patient to considerable radiation as well as involving a long time for the investigation. We therefore prefer isotopes of shorter duration, i.e. ^{99m}Tc, ^{113m}In, which subject the patients lungs to a dose of less than one rad.

CONCLUSIONS

Pulmonary scintigraphy is the method of investigation which is in best agreement with clinical findings.

Routine chest X rays are of limited use in the diagnosis of pulmonary emboli.

The ECG shows characteristic changes only in massive pulmonary embolism.

In contrast to the other methods a measure of the extent of the lesions is obtained through the scintigraphy

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PARTIAL ESCAPE OF MAGNESIUM FROM THE RENAL ACTION OF PARATHYROID HORMONE IN HYPERPARATHYROIDISM

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Abstract. The paradox of the potentially hypermagnesaemic actions of parathyroid hormone (PTH) and the occurrence of hypomagnesaemia in hyperparathyroidism prompted this study of renal handling of calcium and magnesium in hyperparathyroidism. In 15 patients the serum ultrafiltrable calcium (UFCa) and magnesium (UFMg), the urinary excretion of these ions and of sodium, and the clearance of creatinine (CCr), were determined. The fractional reabsorptions of calcium (TRCa%) and of magnesium (TRMg%) were calculated. At levels of CCr above 25 ml/min TRMg% was found to be inversely correlated to UFCa $R = -0.73$, $p < 0.01$) and to urinary sodium excretion per unit of CCr ($R = -0.87$ $p < 0.001$), while no correlation could be established between UFCa and sodium excretion. It is concluded that high concentration of calcium in the ultrafiltrate and high urinary sodium excretion both appear to depress TRMg% enabling normomagnesaemia to be maintained or hypomagnesaemia to develop, despite the enhancing effect of PTH on the tubular reabsorption of magnesium (TRMg).

Current evidence strongly suggests that parathyroid hormone increases the tubular reabsorption of magnesium (5 11 24 31,, enhances intestinal absorption of magnesium (17 25) and accelerates bone resorption with mobilization of magnesium from bone.

These several actions of parathyroid hormone all tend to increase the rate of entry of magnesium into the blood stream. Therefore *hypermagnesaemia* should be a feature of hyperparathyroidism. This is not the case, however as serum magnesium is usually either normal or decreased (15 17 19 22, 34); indeed, the association of hypomagnesaemia with severe hyperparathyroidism (1 3 16, 22, 34) suggests that other effects of increased parathyroid hormone activity supersede those actions of the hormone which tend to increase magnesium concentration in the blood. In recent studies by King and Stanbury (22) and by Sutton (34), comprising large numbers of patients with hyperparathyroidism, an inverse relationship between total serum calcium and total serum magnesium has been demonstrated. Using a crude estimate of the renal clearance of magnesium the first authors also found that hypercalcaemia apparently increased the fraction of filtered magnesium that was excreted.

The purpose of the present study is, by the use of ultrafiltration technique, to assess more directly the filtered loads of calcium and magnesium and the renal tubular reabsorption of the same ions in hyperparathyroidism. At the same time the interference from variations in the urinary sodium excretion on the fractional reabsorption of filtered magnesium is evaluated, since variations in sodium excretion exert a pronounced effect on the renal handling of divalent cations, especially at the lower range of CCr (12, 39).

MATERIAL AND METHODS

Fifteen patients with hypercalcaemia caused by surgically verified hyperparathyroidism (*Table I*) and 5 patients with hypercalcaemia of non-parathyroid origin (Table II) were studied on either 1) standard diet containing approximately 40 mEq calcium, 15 mEq magnesium, estimated from food tables, 36 mM inorganic phosphorus, 60–80 mEq sodium, 50–70 mEq potassium and 1 g protein kg b. wt. (14 patients), 2) high calcium-high phosphorus diet (pat. EW), or 3) ad libitum diet (patients KRC, IWN, ALJ KEO, AJC). All of these patients but one (EKT) have been reported on previously (38, 40).

On average 3–4 determinations of 24-hour clearances of creatinine and of the concentrations of calcium (37 38) and magnesium (29) in serum and ultrafiltrates of serum (38), as well as the 24-hour urinary excretions of calcium and magnesium, were carried out in all 15 patients with

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Presented in part at the VIII Acta Endocrinologica Congress, July 1971 Copenhagen.

Table I. *Average data for calculation and interpretation of the renal handling of calcium and magnesium in 15 patients with hyperparathyroidism*

Pat. no.	Initials	Serum (mEq/l) TOCa	UFCa	TOMg	UFMg	CCr (ml/min)	Urine UCaV (mEq/24 h)	UMgV	TRCa (%)	TRMg (%)	UNaV corr. (mEq/24 h)
Normal range		4.60–5.30	3.28–3.83	1.56–2.03							
1	E. W.	5.40	4.19	1.77	1.35	13	[illegible]	5.1	97.1	79.0	500
2	K. A. E. J.	5.40	3.89	2.02	1.60	20	1.8	5.2	98.4	88.8	385
3	N. W. L.	7.25	5.58	1.94	1.49	39	9.7	3.3	96.9	96.0	154
4	K. R. C.	8.20	6.35	1.28	1.14	44	19.2	5.1	95.2	92.9	150
5	O. L.	7.00	5.29	1.55	1.15	79	20.4	5.2	96.6	96.1	78
6	H. E. T.	5.75	4.15	1.79	1.33	80	14.1	3.1	97.1	98.0	66
7	I. W. N.	5.40	4.18	1.82	1.46	81	13.8	4.0	97.0	97.7	85
8	A. L. J.	5.20	3.98	1.71	1.40	83	14.4	5.5	96.9	96.7	118
9	K. O. E.	6.25	4.70	1.56	1.29	96	14.8	5.8	97.7	96.7	69
10	K. E. O.	5.85	4.48	1.61	1.22	97	13.9	6.3	97.8	96.3	135
11	E. A. M. J.	5.55	4.23	1.89	1.51	101	14.9	4.1	97.6	98.1	47
12	P. S.	6.00	4.38	1.70	1.48	102	15.0	4.7	97.7	97.9	56
13	B. L. M.	5.70	4.35	1.89	1.42	107	15.5	5.2	97.7	97.6	37
14	P. V. N.	5.30	3.92	1.61	1.31	129	14.3	4.0	98.0	98.3	70
15	E. A. M.	5.70	4.30	1.59	1.29	177	20.9	4.2	98.1	98.7	32

$UNaV_{corr}$ = (UNaV (mEq/24 h)/CCr (ml/min)) × 100.

hyperparathyroidism. The same determinations excepting ultrafiltrable magnesium and urinary magnesium were made in the 5 patients with non-parathyroid hypercalcaemia. Tubular reabsorptions of calcium and magnesium were calculated from the values for ultrafiltrable calcium and magnesium (UFCa and UFMg), the 24-hour urinary excretion of these ions (UCaV and UMgV), and the 24-hour clearances of creatinine, and expressed as the percentage of their respective filtered loads (TRCa% and TRMg%) (37).

The 24-hour urinary excretion of sodium (UNaV) was also determined. As the effect of sodium upon the renal handling of calcium and magnesium supposedly depends on the amount of sodium excreted per unit of functional renal mass rather than on the absolute amount excreted per day (6), the latter was corrected to a standard CCr of 100 ml/min ($UNaV_{corr}$) (cf. Table I). Due to the skewed distribution of some variables the assessment of correlation was carried out by the use of the nonparametric Spearman coefficient of rank correlation *R* (8), while Wilcoxon rank sum test for two samples (8) was used for the comparison of the mean values of total calcium (TOCa) and total magnesium (TOMg) in serum in hyperparathyroid and non-parathyroid patients (Table III).

Table II. *Serum total magnesium (TOMg), serum total calcium (TOCa) and the 24-hour clearance of creatinine (CCr) in 5 patients with non-parathyroid hypercalcaemia*

Note the presence of slight to moderate hypomagnesaemia in all patients but the one with the lowest CCr. Simultaneous normalization of TOCa and TOMg was noticed during treatment of sarcoidosis and vitamin D intoxication

Initials	Diagnosis	TOMg (mEq/l)	TOCa (mEq/l)	CCr (ml/min)	Treatment
Normal range		1.56–2.03	4.60–5.30		
EMKR	Collagen disease	1.87	5.93	40	None
		1.60	4.65	66	Spontaneous remission
EKT	Vitamin D intoxication	*1.43*	7.30	48	Vitamin D treatment interrupted
	Postsurgical hypoparathyroidism	1.74	5.15	53	
AFL	Malignant lymphomas	*1.55*	[illegible]	51	None
AJC[a]	Sarcoidosis	*1.22*	5.70	63	None
		1.68	4.88	101	Cortisone 150 mg/d for 10 days
SKI[a]	Collagen disease (?)	*1.22*	5.45	87	None

[a] For additional information see Transbøl et al. (40).

Table III. *Average concentrations (mean ± S.D.) of serum total calcium (TOCa) and total magnesium (TOMg) in hyperparathyroidism (n = 15) and in non-parathyroid hypercalcaemia (n = 5)*

	TOCa (mEq/l)	TOMg (mEq/l)
Controls	4.95 ± 0.18	1.80 ± 0.12
Hyperparathyroidism	6.00 ± 0.85[a]	1.72 ± 0.19[b]
Non-parathyroid hypercalcaemia	6.33 ± 0.89[a]	1.46 ± 0.27[b]

[a] $p > 0.10$. [b] $p < 0.05$.

Summary of abbreviations used

TOCa, Total serum calcium (mEq/l)
TOMg, Total serum magnesium (mEq/l)
UFCa, Ultrafiltrable serum calcium (mEq/l)
UFMg, Ultrafiltrable serum magnesium (mEq/l)
UCaV Urinary 24-hour calcium excretion (mEq)
UMgV Urinary 24-hour magnesium excretion (mEq)
UNaV Urinary 24-hour sodium excretion (mEq)
UN V_{corr} Urinary 24-hour sodium excretion per 100 ml of filtrate/min (mEq)
CCr Clearance of creatinine (ml/min)
TRCa, Tubular reabsorption of calcium (mEq)
TRMg, Tubular reabsorption of magnesium (mEq)
TRCa% Fractional tubular reabsorption of calcium
TRMg% Fractional tubular reabsorption of magnesium

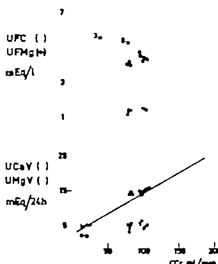


Fig. 1. Relationships between ultrafiltrable calcium and magnesium in serum (UFCa and UFMg), the 24-hour urinary excretion of these ions (UCaV and UMgV) and the 24-hour clearance of creatinine (CCr) in 15 patients with hyperparathyroidism. When the data on UCaV from patients with severe hypercalcaemia are left out (Nos. 3–5), UCaV of the remaining patients is found to be closely correlated to CCr ($R = 0.82$, $p < 0.01$). Contrariwise, UMgV shows no such dependence.

RESULTS

Total and ultrafiltrable calcium and magnesium in serum

In hyperparathyroidism the average TOMg was comparable to that of the control group (Table III). Although borderline or definitely low concentrations of TOMg occurred among three of the four most hypercalcaemic patients (Nos. 4, 5 and 9, Table I), inverse correlations of statistical significance could not be demonstrated between either TOCa and TOMg or UFCa and UFMg. The severity of hypercalcaemia of *the nonparathyroid* group compared well with that of the group of hyperparathyroidism, but the average concentration of TOMg of the former group was significantly depressed (Table III). Four out of five patients in this group had borderline or definitely low concentrations of TOMg (Table II).

Urinary excretion of calcium and magnesium

Average values for each hyperparathyroid patient of UFCa, UFMg, UCaV, UMgV and CCr are shown in Table I and Fig. 1. When three patients with concentrations of UFCa above 5 mEq/l (Nos. 3–5) are left out, UCaV can be evaluated at a fairly constant level of UFCa over the entire range of CCr, 13 to 177 ml/min (Fig. 1). In these 12 patients $UCaV = 0.12 \times CCr + 2.5$, $R = 0.82$, $p < 0.01$. As UFMg and UMgV are both relatively constant, 1.36 ± 0.13 mEq/l (mean ± S.D.) and 4.7 ± 0.9 mEq/24 h, and apparently independent of CCr, these data imply a disproportionately large tubular rejection of magnesium at low levels of CCr.

Relations between CCr, TRCa% and TRMg%

While only a doubtful fall of the TRCa% is noted with decreasing levels of CCr, a steep fall is seen in TRMg% at low filtration rates (Fig. 2).

Relations of the renal handling of calcium and magnesium to serum levels of these ions and to the urinary sodium excretion

At first sight the pronounced dependence of TRMg% on the degree of renal failure made it impossible to establish any correlation between this variable and UFCa ($R = 0$). However, if the

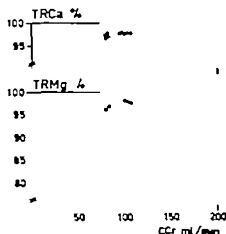


Fig. Relationship between the fractional reabsorption of calcium and magnesium (TRCa% and TRMg%) and the 4-hour clearance of creatinine (CCr). Note the steep fall of TRMg% at low levels of CCr

two patients with the most severe degree of renal failure (cases 1 and _) were left out, a close inverse correlation between TRMg% and UFCa (Fig. 3 A) as well as between TRMg% and $UNaV_{corr}$ (Fig. 3 B) was disclosed. The scatter of TRMg% around the line of regression presented in Fig. 3 A appeared in part to be due to variations in $UNaV_{corr}$ since TRMg% values *above* this line in 10 out of 13 patients was associated with values of $UNaV_{corr}$ *below* the average of 84 mEq _4 h and vice versa. Inspection of Fig. 3 B also suggests that $UNaV_{corr}$ values about 4.5- to 6-fold in excess of the average of the remaining part of the group could explain the excessively low figures for TRMg% in cases 1 and 2.

In the same group of 13 patients $UNaV_{corr}$ also appeared to influence TRCa% but to a much lesser extent ($R = -0.58$, $p < 0.05$). Assessment of the correlation between the remaining pairs of variables, TRMg% and UFMg, TRCa% and UFCa, $UNaV_{corr}$ and UFCa, and CCr and UFCa, gave R-values of no statistical significance ($p > 0.10$).

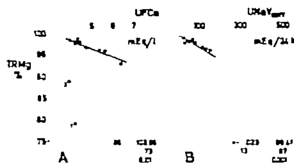


Fig. 3. Correlations between (A) the fractional reabsorption of magnesium (TRMg%) and serum ultrafiltrable calcium (UFCa) and (B) TRMg% and the amount of sodium excreted per unit of functional renal mass ($UNaV_{corr}$). T patients (cases 1 and 2) with the most severe degree of renal failure and extremely low values of TRMg% were not included in the calculation of the regression equations. (R = the non-parametric Spearman coefficient of rank correlation.)

DISCUSSION

The differences in the pattern of calcium and magnesium reabsorption and excretion shown in Figs. 1 and _ are qualitatively similar to those observed in primary renal disease (6, 30, 33). In both conditions, at the lower range of CCr the curves are most likely shaped by the competing effects of high circulating levels of parathyroid hormone and the obligatory solute diuresis (6). Although correlation does not necessarily indicate causality the amount of sodium excreted per unit of functional renal mass appeared to exert a general influence upon TRMg% (Fig. 3 B). What ever the mechanism may be, this relation represents a serious hindrance when assessment of the effect of hypercalcaemia per se is intended. Accordingly like King and Stanbury (__) we had to leave out patients with clearances of creatinine below 25 ml/min, and by use of this precaution TRMg% was now found to be closely and inversely related to UFCa (Fig. 3 A). Furthermore, since the scatter of TRMg% around this line of regression seemed in part to be due to variations in sodium excretion, this correlation is probably even closer than indicated by the correlation coefficient (R) of -0.73 ($p < 0.01$).

Thus it appears that increasing levels of UFCa, directly or indirectly depress TRMg and thereby tend to decrease serum magnesium. Considering the varying contributions to serum magnesium from intestinal and osseous sources it is readily understood why the inverse correlation between TOCa and TOMg can be demonstrated with certainty only in larger series of patients (__, 34). The same consideration applies to the lack of statistical significance of the correlation between UFMg and TRMg%.

If the interpretation of our observations is cor

rect, hypomagnesaemia should also be a feature of non-parathyroid hypercalcaemia. As a matter of fact the minimal levels of circulating parathyroid hormone, supposedly present in these conditions, should facilitate the development of hypomagnesaemia. Unfortunately the paucity of data reported and the high incidence of severe renal failure in these conditions (40) make a proper evaluation difficult. Nevertheless hypomagnesaemia has been reported in vitamin D intoxication in rats (1 28) in dogs (10) and in normal persons (9 23), as well as in a few cases of cancer of the breast and myelomatosis, being associated with hypercalcaemia. Admittedly 3 cases of hypercalcaemic sarcoidosis with normomagnesaemia have also been reported (8 21).

Table II supplements these data with our own experience. Slight to moderate hypomagnesaemia was found in four out of five instances of sarcoidosis, vitamin D intoxication malignant lymphoma and collagen disease, and simultaneous disappearance of hypercalcaemia and hypomagnesaemia was also noticed in the two first-named conditions. The fact that the average concentration of TOMg in these patients was significantly lower than in the group of hyperparathyroidism (Table III) is consistent with our interpretation, since in these disorders hypercalcaemia actually exerts its effect on TRMg% unopposed by parathyroid hormone.

The nature of the mechanism by which hypercalcaemia depresses the tubular reabsorption of magnesium is unknown, and several theories have been advanced. One of these is based on the frequent association of hypercitricaemia with hypercalcaemia and implies consequently an increased complex-binding of magnesium by citrate in the tubular urine (4 9). This theory has neither been supported nor rejected by our study or by others. The theory of Sutton (34), claiming that hypercalcaemia occasionally causes a tubular defect of magnesium reabsorption in analogy with the rare abnormality of potassium loss, disregards the obvious effect of renal failure as such upon magnesium conservation.

Although only a few interrelations between magnesium and calcitonin have been established as yet, the effect of hypercalcaemia on TRMg% could be mediated through hypersecretion of calcitonin. The current evidence for calcitonin-magnesium interactions includes in vitro stimulation of calcitonin secretion by magnesium (4) and a hypomagnesaemic effect of calcitonin (3 7 32, 35). So far only one group has successfully demonstrated a hypermagnesuric action of calcitonin (32). However very recent observations by Tashjian et al. (36) show that sustained hypersecretion of calcitonin is probably *not* a common feature of chronic hypercalcaemia.

Finally the relationship could be explained by a competition between calcium and magnesium for a common reabsorptive mechanism. Such competition is often inferred from infusion studies showing enhancing effects of calcium and magnesium on the respective urinary excretion of magnesium and calcium, but this interpretation is open to debate. The bulk of such studies implicate either the use of salts, such as calcium gluconate and magnesium sulphate, the anions of which are not readily reabsorbable (20 41) or the use of sodium chloride as a vehicle for the calcium or the magnesium salt (41). Certainly the few studies in which calcium chloride (2) and magnesium chloride (6, 42) have been used reveal enhancing effects on the respective urinary excretion of magnesium and calcium, but calcium and magnesium both inhibit secretion of parathyroid hormone and may cause decreased reabsorption of both ions by this mechanism alone. Admittedly Coburn et al. (7) have shown that infusions of calcium chloride decrease the fractional reabsorption of magnesium in thyroparathyroidectomized dogs, but to our knowledge no data have been presented on the effects of magnesium chloride in hypoparathyroidism. Nevertheless, although it remains to be established, competition appears to be the most likely explanation of our observations.

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Acta med. scand. Vol. 193 pp. 331–336, 1973

THE CALCIUM INFUSION TEST IN PRIMARY HYPERPARATHYROIDISM

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Abstract. The calcium infusion test as described by Kyle et al. (10), which involves measurement of forenoon phosphate clearance *before and after* calcium *infusion*, has been performed in 10 patients with primary hyperparathyroidism (PHP), 4 with non-parathyroid hypercalcemia and in 12 control patients together with 11 patients with other diseases (kidney stone, peptic dyspepsia, and normocalcemic sarcoidosis). The fall in phosphate clearance (F%) indicated the degree to which the parathyroid glands could be suppressed. F% did not completely differentiate patients with PHP from normocalcemic patients because of a marked overlap. Only values less than 10% were indicative of PHP. In patients with non-parathyroid hypercalcemia a low F% was also found and was dependent upon the degree of hypercalcemia. In normocalcemic patients F% was found to be dependent upon the increase in serum calcium concentration which could be achieved during infusion. This correlation was not found in patients with PHP. No relationship was found between the degree of adenoma activity expressed by the serum calcium concentration, and F%. Whether forenoon phosphate clearance was suited as a criterion for differential diagnosis of PHP was also studied. This did not appear to be the case.

A whole series of calcium infusion tests have been developed as diagnostic aids with regard to primary hyperparathyroidism (PHP). All of these tests are dependent upon the fact that normal hormone secretion from the parathyroid glands is subject to negative feedback regulation controlled by the ionized serum calcium and the phenomenon that parathyroid tumors have an autonomic secretion which cannot be suppressed after i. v. administration of calcium salts.

Various parameters have been used as a measurement of parathyroid function before and after calcium infusion. Howard et al. (9) used the rise in serum phosphate after infusion together with the fall in the 24-hour excretion of phosphate on the day of infusion. Pronove and Bartter suggest a modification (17) which employs the compensatory rise in the 24-hour excretion of phosphate on the day after infusion. Bell et al. (3) used both the 24-hour excretion of phosphate and hydroxyproline on the day of infusion and on the day before. These procedures have, however, been proven to be unsuited (6, 7, 14, 19, 20) because there is a marked overlap between patients with PHP and normal control individuals. Kyle et al. (10) described a method in which the phosphate clearance (Cl_{ph}) is determined in the forenoon before and after the infusion of calcium and the percental fall of phosphate clearance is calculated. They found that this procedure was able completely to differentiate patients with PHP from normal controls. These findings have been reproduced by Haas (6, 7).

In the present study we have attempted to evaluate the usefulness of the procedure of Kyle et al. in diagnosing PHP. We have also studied whether Cl_{ph} measured as forenoon clearance was suited as a criterion for differential diagnosis of PHP.

MATERIAL

Thirty-seven patients admitted to the Medical Department of Arhus County Hospital during the period 1969–71 were studied. The patients were divided into the following four groups.

Group 1. Ten patients with PHP. The diagnosis was verified by operation, a solitary parathyroid adenoma being found in all.

Group 2. Four patients with non-parathyroid hypercalcemia. One had cancer of the breast with bone metastases, another a phaeochromocytoma with hypercalcemia, which disappeared after operative removal of the phaeochromocytoma. A third patient had a biopsy-verified Boeck's sarcoidosis with steroid suppressible hypercalcemia, and the fourth had hypercalcemia as part of an Addison crisis.

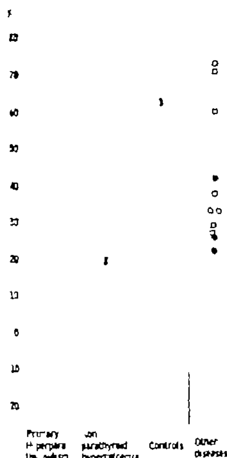


Fig. 1. Fall in Cl_{Ca} expressed as $F\%$ after calcium infusion in all 37 patients studied. The group of patients with other diseases consisted of 4 patients with nephrolithiasis (○), 3 with Boeck's sarcoidosis (○), and 4 with peptic dyspepsia (□).

Group 3. Nine patients with diseases with no relationship to calcium metabolism and three healthy students. Twelve in all.

Group 4. Eleven patients with diseases which may present differential diagnostic problems with regard to PHP. This group included four patients with nephrolithiasis, four with peptic dyspepsia and three with normocalcemic sarcoidosis.

METHODS

A calcium infusion test as described by Kyle et al. (10) was performed in all patients.

Three days before the test the serum calcium concentration was determined daily. On the basis of these values a mean value was determined, which was employed in the following calculations. During the study and for two days preceding it the patients were given a normal diet together with 1/2 l milk daily in order to secure an adequate phosphate administration. Patients were kept on complete bed rest during clearance measurements, but during other periods they are not confined to bed. On day 1 the Cl_{Ca} and the creatinine clearance (Cl_{cr}) are determined between 8 a.m. and 10 a.m. On the evening of the same day between 8 p.m. and 11 p.m. 10 mg Ca/kg b.wt. was given intravenously in the form of calcium levulinate in 500 ml 0.9% sodium chloride. At 11 p.m. the serum calcium concentration was determined. As a criterion of good suppression a rise of at least 0.50 mmol/l in the serum calcium concentration in relation to the above mentioned mean value was considered essential. On day 2 clearance measurements were repeated during the same periods as mentioned above. During the clearance measurements and two hours previously the patients were given 2.4 l fluid by mouth in order to achieve an adequate urinary flow. Voluntary bladder emptying was employed after this had been found to be sufficient on the basis of urography. In order to eliminate any possible defects in the collection of urine, Cl_{Ca} was expressed in relation to Cl_{cr} according to the following formulae:

$$f-1 = \frac{Cl_{Ca} - \text{1st day}}{Cl_{cr} - \text{1st day}}$$

$$f-2 = \frac{Cl_{Ca} - \text{2nd day}}{Cl_{cr} - \text{2nd day}}$$

The fall in the corrected Cl_{Ca} was thereafter expressed by

$$F_{\%} = \frac{(f-1)-(f-2)}{f-1} \cdot 100$$

Calcium was determined by flame photometry (Eppendorf flame photometer). Serum protein level was determined simultaneously with the serum calcium also as a control measure with regard to the blood sampling technique. Phosphate was determined using the molybdenum blue method (18). Creatinine was determined using the Jaffé reaction after absorption with Lloyd' reagent.

RESULTS

F% values for the four patient groups are given in Fig. 1. A F% <20% was seen in 6 of 10 patients with PHP, in 3 of 4 patients with non parathyroid hypercalcemia, and in of 12 control patients. F% <20% was not observed in the group of patients with other diseases. There was no statistically significant difference between the groups. The group of patients with PHP was then compared with the total group of normocalcemic patients, i.e. the control group and the group of patients with other diseases. A F% <20% was found in 6 of the 10 and in 2 of the 23 patients, respectively. This difference was statistically significant (Fischer's exact test, $2\alpha = 0.02$).

Thus measurement of the F% could not be used to differentiate the PHP group from the non parathyroid hypercalcemia group but could, on the other hand differentiate PHP pa-

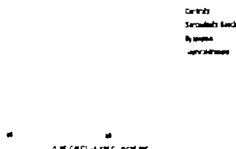


Fig. 2. Correlation between F% and the rise in serum calcium concentration (mmol/l) after calcium infusion in 23 normocalcemic patients.

tients from normocalcemic patients. There was, however, an overlap of results between the two latter groups of patients, so that only very low F% values (i.e. < 10%) appeared to suggest the diagnosis of PHP.

It should also be mentioned that there was some relationship between F% and the rise in serum calcium concentration (Δserum calcium concentration) after infusion. As show in Fig. 2, there was connection between F% and the rise in serum calcium concentration in normocalcemic patients, there being a greater rise in serum calcium the greater the F%. This relationship was statistically significant (Spearman's rank correlation, $R = 0.49$, $2\alpha = 0.02$). In patients with PHP a corresponding relationship could not be demonstrated.

Based on the assumption that serum calcium values in individual patients with PHP are an expression of the activity of the parathyroid adenoma, the relationship between F% and the mean concentration of serum calcium was studied in patients with PHP. No significant statistical correlation was found between these two parameters (Spearman's rank correlation, $2\alpha > 0.10$).

Table I. *Correlation between serum calcium concentration and F% in four patients with non-parathyroid hypercalcemia*

Pat. no	Mean serum calcium (mmol/l)	F%
1	2.73	20
2	2.75	19
3	2.87	9
4	2.98	0

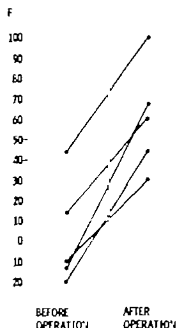


Fig. 3. F% before and after operative removal of a parathyroid adenoma in 5 patients with PHP.

As shown in Table I, it is possible that a correlation between F% and mean serum calcium concentration is to be found in patients with non-parathyroid hypercalcemia, increasing serum calcium values being accompanied by falling F%.

In 5 of 10 patients with PHP a calcium infusion test was carried out as well preoperatively as postoperatively. The postoperative study was performed 2–9 months after operation. As shown in Fig. 3 a higher F% value was found postoperatively in all five patients. There was, however, an overlap of preoperative and postoperative values. In one patient the postoperative Cl_{ph} after calcium infusion was 0 and the F% therefore 100%.

In normal individuals phosphate excretion varies during the day, being lowest during the morning. This diurnal rhythm is not seen in PHP (10, 13, 16). We therefore investigated whether Cl_{ph} measured between 8 and 10 a.m. was higher in PHP patients than in other patients. As shown in Fig. 4, high forenoon Cl_{ph} values were seen more commonly in patients with PHP than in normocalcemic patients. $Cl_{ph} > 20$ ml/min was found in 5 of 10 patients with PHP as against 1 of 5 patients with non-parathyroid hypercalcemia. These differences were not statistically significant (Fischer's exact test, $_{2}\alpha = 0.10$, $_{2}\alpha = 0.20$).

In five patients with PHP studied both preoperatively and postoperatively the lowest forenoon Cl_{ph} was in all cases found after removal of the parathyroid adenoma without, however, there being any absolute separation (Fig. 5).

DISCUSSION

In a study of 10 patients with PHP and 11 normal control patients Kyle et al. (10) found a complete separation between the groups. All normals demonstrated a fall in Cl_{ph} of more than 40% whereas none of the PHP patients had a fall above 25%. Haas (6, 7) was also able to show complete separation between 25 patients with PHP and 24 control patients, including normals, patients with kidney stones, and patients with hypercalcuria with and without kidney stones, as

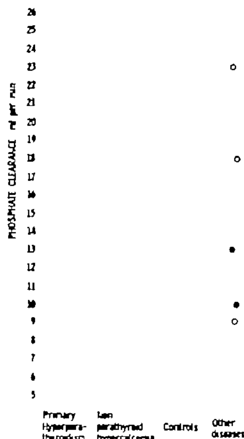


Fig. 4. Forenoon Cl_{ph} measured between 8 and 10 a.m. in all 37 patients studied. Nephrolithiasis, Boeck's sarcoidosis and peptic dyspepsia indicated as in Fig. 1.

well as between 11 patients with PHP before and after operation. The maximal fall in Cl_{ph} after calcium infusion was found to be 25% for patients with PHP whereas all control patients had a fall greater than 25%.

In the present study using the same procedure, a marked overlap was found between the various F% values in patients with PHP control patients, patients with non-parathyroid hypercalcemia, and a group of patients with normocalcemic sarcoidosis, peptic dyspepsia, and kidney stones. More patients with PHP had, however F% <20% than did normocalcemic patients, and F% <10% was not seen in any normocalcemic patient. All patients with non-parathyroid hypercalcemia had rather low F% values (≤20%), corresponding to the fact that the parathyroid glands in these patients were already endogenically suppressed. Kyle et al. have reported the same finding (10). A possible relationship was found between the degree of hypercalcemia before calcium infusion and the F%. Thus F% values less than 10% suggest the diagnosis of PHP if the patient is normocalcemic or if other causes of hypercalcemia can be excluded.

F% in normocalcemics was found to be dependent on the increase in serum calcium after calcium infusion. This increase is dependent on a series of individual values such as calcitonin production and the degree of adiposity. Ca^{++} 10 mg/kg b.w.t. was given intravenously to all patients. In 3 lean individuals in the control group it was impossible to achieve the required increase of 0.50 mmol calcium/l and thus these patients were excluded from the final analyses. In order to achieve a more uniform suppression, one should perhaps administer i.v. calcium according to lean body mass instead of body weight.

Several patients with PHP could be suppressed with a calcium infusion. Parathyroid adenomata with only slight activity and intermittent slight hypercalcemia could perhaps appear together with normally suppressible parathyroid glands, where as normal parathyroid glands are completely suppressed endogenically in patients with active adenomata accompanied by marked hypercalcemia. In the present study however no relationship was found between the degree of hypercalcemia and F% in patients with PHP.

In all patients studied a marked increase in F% was found postoperatively as compared to

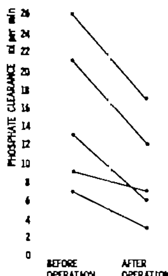


Fig. 5. Forenoon Cl_{ph} measured between 8 and 10 a.m. before and after operative removal of parathyroid adenoma in 5 patients with PHP.

preoperative values, suggesting that the excised adenomata were completely autonomic. In contrast to Haas (6, 7) no complete separation was seen between preoperative and postoperative values.

As demonstrated by several investigators (1, 6, 11), Cl_{ph} measured on the basis of a 24-hour clearance period has only a limited value in the diagnosis of PHP. Since Cl_{ph} in normals is lowest during the morning and this diurnal variation is not seen in patients with PHP, the forenoon clearance of phosphate was therefore evaluated with regard to its suitability as a differential diagnostic criterion, but with negative results.

The F% value used, which was corrected for any possible defect in urine collection, did not differ markedly from the non-corrected fall in Cl_{ph}. This can be taken as evidence that urine collection was adequate.

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Table I *Composition of the matched series*

Matching feature	Diabetics Pregnant	Diabetics Non-pregnant	Non-diabetics Pregnant	Non-diabetics Non-pregnant
Mean age (y)	25.6	25.5	25.4	25.4
Mean duration of diabetes (y)	8.6	9.7		
Parity number in no. of pats.				
1	58		56	
2	46		47	
3	20		23	
≥4	8		7	
Mean gestational week of examination	26.1		27.0	

pregnant women and were collected consecutively during the period 1.8.1966–1.8.1967 (3). After excluding subjects who developed diabetes mellitus or glycosuria during pregnancy the subjects were matched with patients in material A with respect to age, marital status, number of previous deliveries and gestational age at the time of the investigation.

D. *Non-diabetics—non-pregnant*

Subjects falling into this group had to satisfy at least two of the following three criteria: postprandial venous blood sugar value less than 130 mg/ml, no glycosuria and no history of diabetes mellitus in the family. Pregnancy was excluded solely on the basis of information as to menstruation. The subjects were in part those from previous material of casualty ward patients with minor lesions (8), in part other outpatients. They were matched with material A on the basis of age and marital status.

In no group were antibiotics given one week prior to the day of investigation. Table I shows the distribution and comparability of the different groups.

METHODS

After prior thorough instruction, midstream urine was collected from all patients (9, 10), immediately cooled to +4°C and transported at this temperature to the laboratory (10). Here the samples were examined quantitatively by two different methods: 1) logarithmic series dilution to 10^{-4} with inoculation of 0.5 ml onto three blood agar plates as described previously (9), 2) inoculation by calibrated loops onto both blood agar and lactose plates containing bromothymol blue (10). A good agreement was found between the two methods.

Bacterial strains present to an amount of more than 100 000 colonies/ml urine were isolated, subcultured and identified by means of the usual biochemical reactions.

Much of the bacteriological work was done by the author and a minor part by trained laboratory assistants under the author's control.

The history was obtained by the author.

Glucose in urine was determined qualitatively by Clinistix®.

The statistical analysis was done using the χ^2-test with Yates correction and 1 degree of freedom. The comparison between non-diabetics and non-pregnant diabetics, however, was made by Fisher's exact test. Neither of these tests takes into account that the material is matched, with the result that the p values found may be too high, so that the actual differences are underestimated.

Definitions

Significant bacteriuria, at least 100 000 colonies/ml urine.

Age: the chronological age on the first day of investigation.

Duration of diabetes: number of years from the diagnosis of the disease until the day of investigation.

RESULTS

A. *Diabetics—pregnant*

In the 2-year period altogether 172 patients were recorded, three of whom were found not to be pregnant and four not to have diabetes mellitus on closer examination. Nine patients were repeat cases in the material. Twenty-four underwent abortus provocatus on medical indications; only one of these 24 had significant bacteriuria. The total material consists of 132 patients. Their mean age, mean duration of diabetes and parity appear from Table I.

A grouping on the basis of White's classification (13) gave 16 in group A, 30 in group B, 29 in group C, 51 in group D and 6 in group F.

No case in the material, either at the time of investigation or prior to it, had received analgesics in large amounts, four had received antibiotics early in pregnancy for a presumed infection of the urinary tract, three of them had significant bacteriuria at the time of the investigation.

Apart from the manifestations of late diabetes, as indicated by the White classification, there were only few cases of complications. Among other diseases than diabetes, there was disease of the thyroid in nine cases (atoxic small struma), and pulmonary disease, neurological disease, heart disease and disease localized to the intestinal tract, each in one patient. Of the total of 13 patients with another disease in addition to diabetes, only one had significant bacteriuria. Eleven patients were unmarried, 112 were married and nine were divorced. An attempt to classify the material socially on the basis of occupation showed that 76 were engaged in housework alone, 36 had half

day jobs of a light nature, 20 were occupied with whole-day jobs. The geographic distribution showed that 85 of the patients lived in a large town, 35 in small provincial towns and 12 in country districts.

Time of investigation. Eighteen patients (14%) were examined for the first time during the 1st trimester. 30 (23%) during the 2nd, and 82 (62%) during the 3rd. Between 1 and 7 cultures were made from the urine in each patient (a er age 2.7 cultures/patient) 28 (21%) had only 1 urine culture made. Cultures were made more than twice in all patients with significant bac teriuria. Three patients developed significant bac teriuria during the period of observation. They are included in the material at the time of commencement of the bacteriuria, and the control subjects were chosen in accordance therewith. Six patients who had significant bacteriuria initially and who subsequently received treatment for it are also included in the material. The distribution of significant bacteriuria was not cor related with the marital status of the patients, work or residence.

B. *Diabetics—non-pregnant*

In the two periods 128 (8) and 138 patients, respectively were collected, with 132 matched controls selected consecuti ely in accordance with the criteria mentioned above. Table I shows the mean age and the duration of diabetes.

C. *Non-diabetics—pregnant*

From the consecuti ely collected material of 2 122 patients, 132 were selected on the criteria *mentioned above (3).*

The distribution with respect to mean age and parity as well as the mean gestational age on examination, are shown in Table I.

D *Non-diabetics—non-pregnant*

From the casualty ward and outpatient clientele of about 300 subjects, 132 control subjects were selected consecutively

The ge distribution is seen in Table I.

Incidence of bacteriuria

The incidence of significant bacteriuria in the respective groups at the time of investigation, the bacteriuria being established by one quantitati determination, appears from Table II. Significant

Table II. *Prevalence of bacteriuria in the matched series*

Col/ml urine	Diabetics				Non-diabetics			
	Pregnant		Non-pregnant		Pregnant		Non-pregnant	
$<10^5$	108		117		126		126	
$>10^5$	24	18.2	15	11.4	6	4.5	6	4.5
Total	132		132		132		132	

bacteriuria is seen to occur considerably more frequently in diabetic than non-diabetic subjects, and this difference increases with the onset of pregnancy. In the present material a greater incidence of bacteriuria in pregnant non-diabetic subjects, in comparison with non-pregnant, non-diabetic subjects, cannot be demonstrated.

Using the χ^2-test, a possibly significantly" greater incidence of bacteriuria was found in non-pregnant diabetics by comparison with non-pregnant non-diabetics ($0.1 > p > 0.05$). The difference between diabetics and non-diabetics is more pronounced and statistically significant ($p < 0.001$) at the onset of pregnancy. The incidence of bacteriuria in pregnant diabetics is not significantly greater statistically than in non-pregnant diabetics ($p > 0.1$). Whether this signifies that there is no difference between pregnant and non-pregnant diabetics, or whether the material is simply not extensive enough to identify an ac tual difference, cannot be decided. Thus, if it is desired to determine whether the incidence of bacteriuria rises from the approximately 12% in non-diabetics to the approximately 18% or more in the pregnant subjects, the material must consist of at least 762 patients in each of the two groups, i.e. it would have to be about 6 times as extensive as the present material (1-sided test, $\alpha = \beta = 0.05$)

Isolated bacterial strains

The distribution of the isolated bacterial strains in the patient groups is shown in Table III.

In all groups *E coli* is the commonest micro-organism isolated when counts are $> 10^5$ colonies/ml. For counts $< 10^5$ colonies/ml no differences was found between the groups. Here, mainly

Table III. *Isolated strains f om patients with >10⁵ col /ml urine in the four matched series*

	Diabetics		Non-diabetics	
Organism	Pregnant	Non-pregnant	Pregnant	Non-pregnant
E. coli	18	10	5	4
Klebsiella spp.	1	1		1
Citrobacter spp.	1			
Staph. albus	3		1	1
Staph. aureus		3		
Strept. faecalis	1	1		
Total	24	15	6	6

staphylococci and coryneform rods were found, as well as a few cases with lactobacilli and non-identified Gram-negative rods. While mixed cultures occurred frequently in patients with $<10^5$ colonies/ml, this was only the case in one patient with $>10^5$ colonies/ml urine in whom an *E coli* strain was found together with a *Strept faecalis* strain.

DISCUSSION

Only few studies of bacteriuria in pregnant diabetics have been published previously Pometta et al. (6) examined 253 pregnant diabetics who were screened for bacteriuria on their first at ance at the Joslin Clinic. Most of the patients examined four or five times during the of pregnancy The occurrence of significant bacteriuria, evaluated in half of the patients by the first two cultures with $>10^5$ colonies/ml urine, and in the other half by one culture with $>10^5$ colonies/ml urine, was 7% As far as can be judged, only cultures with Gram-negative rods were included among the positive cases. The above investigators compared this incidence with two non-matched series, partly from the same and partly from another hospital, and found, respectively 4% and 7% with significant bacteriuria in pregnant diabetics. The authors suggested on the basis of these results that significant bacteriuria might occur more frequently in pregnant diabetics.

Later Bruns et al. (1) examined 573 pregnant diabetics and compared them with 445 non-pregnant diabetics, 89 pregnant non-diabetics and 137 non-pregnant non-diabetics. The incidence of patients with $>10^5$ colonies/ml urine was, respectively 12.8% 9.7% 8.6% and 5.0% No further details are given for the selection the criteria or the times of investigation, but it is noted that the growth of *Staph. epidermidis* $>10^5$ colonies/ml is not regarded as significant bacteriuria.

In the present study significant bacteriuria was found with greater incidence in the group of pregnant diabetics than in the latter studies. A comparison with these studies is difficult, particularly because there is only limited information on the composition of the material, especially with regard to the choice of the control groups.

In the present investigation the patients have been continuously observed over a lengthy period by the same investigator and in selecting the control groups close attention has been paid to a number of factors which, as experience has shown may cause changes in the incidence of bacteriuria.

The reason for the higher bacteriuria incidences than in other studies may be due to the fact that the average duration of diabetes is longer and that the material contains a preponderance of patients in White's groups D and F (11).

The definition of significant bacteriuria employed in the present study (1 culture $>10^5$ colonies/ml urine) may have contributed to higher values than if the choice had been two consecutive cultures with $>10^5$ colonies of the same micro-organism/ml urine which would have increased the statistical certainty to 95% (7) However this holds only for the control groups, as subsequent cultures made in the pregnant diabetics have confirmed the positive findings.

For practical reasons it has not been possible to establish the requirement of two consecutive studies for all groups. The micro-organisms isolated in the present study do not differ from those found by others (11) but so-called apathogenic staphylococci (*S. epidermidis, S. albus*) in findings $>10^5$ colonies/ml urine, are here included among cases with significant bacteriuria and therefore contribute to an increased incidence of significant bacteriuria.

The smaller incidence of significant bacteriuria found in the present study among non-pregnant diabetics than in a previous study (8) may be due to the lower mean age and the briefer duration of diabetes in the present study While the

present study does not appear to show a difference in incidence of bacteriuria between pregnant and non-pregnant non-diabetics, diabetics show an increasing incidence of bacteriuria with the onset of pregnancy The supposition that pregnancy per se influences the incidence, however cannot be evaluated more exactly in the present small material, but must await more extensive, well controlled investigations which are not cross-sectional but longitudinal. The observations suggest that pregnancy per se does not determine the development of bacteriuria, but is the condition in which bacteriuria is demonstrated.

ACKNOWLEDGEMENTS

This study was aided by grants from the Novo Foundation and the F L. Smith Foundation.

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Acta med. scand. Vol. 193 pp. 343–346, 1973

STUDIES ON URINARY INFECTIONS IN DIABETICS

IV. Significant Bacteriuria in Pregnancy in Relation to Age of Onset, Duration of Diabetes, Angiopathy and Urological Symptoms

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Abstract. A material consisting of 132 non-selected pregnant diabetics has been investigated, employing two consecutive urine cultures giving $>10^5$ colonies of the same micro-organisms/ml urine as criterion of the presence of urinary infection. The findings were as follows: 1) Significant bacteriuria at more than 1 investigation was present in 18.2% of the patients (24 out of 132). Twenty-one of the patients presented with significant bacteriuria, *three developed bacteriuria later in pregnancy. 2) The oc*currence of urinary infection could not be correlated to *increasing age. 3) On the other hand there was sig*nificantly increasing incidence of urinary infections in patients with duration of diabetes. 4) A statistically significant correlation was found between the presence of diabetic angiopathy expressed as the occurrence of diabetic retinopathy and the incidence of urinary infection. No attempt was made to correlate the incidence of significant bacteriuria to other forms of angiopathy as the material was too small and the criteria inadequate. 5) Urinary infections occurred with significantly greater incidence in White's groups D and F than in A, B and C (18 out of 57 against 6 out of 75). 6) Of the cases with demonstrated urinary infection 62.5% were asymptomatic and ran an asymptomatic course throughout pregnancy. 7) Approximately 25% of the patients with dysuria and pollakisuria did not have significant bacteriuria. 8) The relationship between urinary infection and diabetic angiopathy appears to have been further emphasized. The desirability is pointed out of an investigation of the possible relationship between subclinical neuropathy of the bladder and significant bacteriuria.

The author has previously demonstrated an increased incidence of significant bacteriuria in pregnant diabetics (7). Based on the same material, the present article elucidates significant bacteriuria in relation to clinical data in pregnant diabetics. In particular, the possible relationship of bacteriuria to angiopathy of late diabetes has been evaluated; this relationship has been discussed previously (1, 3, 4, 6).

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MATERIAL AND METHODS

The material and methods employed have been described in detail elsewhere (7).

The material consisted of 132 non-selected pregnant diabetics with mean age 25.6 years and mean duration of diabetes 8.6 years. The patients were followed by the author approximately every 14 days, by means of both quantitative and qualitative urine cultures from the time of first attendance at the Diabetes Centre until delivery. (The first attendance took place on an average at 26 weeks.)

The evaluation of the eyegrounds for the presence of possible retinopathy was repeated several times in the same patient by doctors from the Ophthalmological Department of the University Hospital.

A White grouping (8) was carried out by J. Pedersen. The χ^2-test with Yates correction was used in the statistical analysis.

Definitions

Significant bacteriuria. Two consecutive cultures with $>10^5$ colonies of the same micro-organisms/ml urine.

Diabetic retinopathy. The criteria in evaluating diabetic retinopathy are as follows (6). *Grade 0:* normal ophthalmoscopy. *Grade 1:* few scattered microaneurysms, no haemorrhages or exudates. *Grade 2:* microaneurysms, haemorrhages and exudates. *Grade 3:* progressive proliferative retinopathy.

White's classification (8). Group A: diabetes treated with diet alone. *Group B:* insulin-treated diabetes diagnosed at the age of 20 or later, with no late diabetic complications, in particular no retinopathy. *Group C:* diabetes diagnosed before 20 years of age, nearly always in the age group 10–19, and with no late diabetic complica-

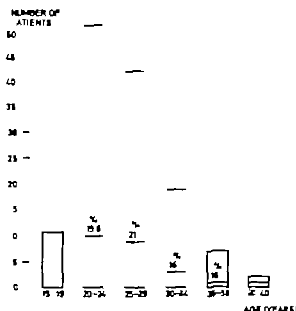


Fig. 1. Distribution of diabetics according to age (□) and incidence of urinary infection in relation to age (▭).

tions. *Group D*: diabetes with non-proliferative retinopathy. *Group E*: not used (calcification of pelvic arteries). *Group F*: vascular nephropathy and/or proliferative retinopathy.

Age and duration of diabetes are defined as indicated before (7).

RESULTS

Incidence of bacteriuria

As already shown, 24 of the 132 patients (18.2%) had significant bacteriuria at more than one investigation. Twenty-one patients had significant bacteriuria at the first examination, while three developed bacteriuria during the period of observation. Nine patients were treated on the basis of symptoms of dysuria and pain in the loins, a few had also a rise in temperature. Four of these nine patients had a recurrence. Treatment was withheld in the remainder of the 24 patients on account of the investigation.

Age

Fig. 1 shows the distribution of the material with respect to age and incidence of significant bacteriuria. This material of preponderantly younger diabetics shows no increasing incidence of significant bacteriuria with respect to age.

Duration of diabetes

Fig. 2 shows the distribution of the patients with respect to the duration of diabetes mellitus, as well as the number of patients with significant bacteriuria in relation to duration of diabetes. An increasing incidence of significant bacteriuria is seen with increasing duration of diabetes, and if the material is grouped into two lots at a duration of ten years, the difference is statistically significant (5/72 in the group with shorter duration of disease, 19/60 in the group with longer duration of disease, 6.9% and 31.7% respectively $p < 0.001$). A non-parametric rank correlation test also shows that the incidence of significant bacteriuria increases in statistical significance with increase of the duration of diabetes ($p < 0.001$).

Age at onset of diabetes

In view of these findings the incidence of significant bacteriuria must be presumed to be greater in cases with earlier onset of the disease. This assumption is confirmed in Fig. 3 as a non-parametric rank correlation test shows a statistically significant relationship between incidence and duration ($p < 0.001$).

Diabetic retinopathy

Table I shows the distribution of the material within the various grades of retinopathy together with the percentage distribution of significant bacteriuria.

The occurrence of bacteriuria appears to increase with increasing grade of retinopathy al-

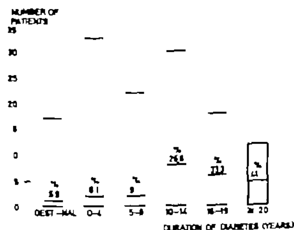


Fig. 2. Distribution of diabetic series according to duration of diabetes (□) and incidence of urinary infection in relation to duration of diabetes (▭).



Fig. 3. Distribution of the material according to age at onset of diabetes (□) and incidence of urinary infection in relation to age at onset of diabetes (▨).

though this increase is not statistically significant when evaluated by a non-parametric rank correlation ($0.1 > p > 0.05$)

If the group without ophthalmoscopic changes is compared with the group showing retinopathy grades I and II, the incidence of the bacteriuria is significantly greater ($p < 0.05$) in patients with angiopathy (χ^2-test with Yates correction, d.f. = 1).

Other angiopathic phenomena of diabetes

The restricted nature of the present material does not permit an elucidation of a possible relationship between these phenomena and bacteriuria. It

Table I. *Urinary infection in relation to grade of retinopathy*

Grade of retinopathy	No. of pats.	Patients with significant bacteriuria	
0	87	11	12.6
I	4	6	25 0
II	20	7	35 0
III	1	—	—
Total	132	24	—

might be mentioned, however that five of the patients had constant albuminuria, three of whom had significant bacteriuria in addition two patients had serum creatinine values greater than 1 2 mg% both of them with >10 colonies/ml urine. It may be added that three of the patients had a BP value above 140/90 in the recumbent position, two of whom had bacteriuria.

White groups

Table II shows the distribution of the material with respect to White s classification (8) together with the relationship to significant bacteriuria. As expected, most of the cases of bacteriuria are found in groups D and F the incidence here being significantly greater than in groups A+B+C (18/57 6/75 8.0% 31 6% $p < 0.005$) In addition a non-parametric rank correlation test confirms the relationship between bacteriuria and the White classification ($p < 0.01$)

Table II. *Urinary infections in relation to White s classification*

White group	No. of pats.	Patients with significant bacteriuria	
A	16	1	
B	30	3	
C	29	2	
A+B+C	75	6	8 0
D	51	14	
F	6	4	
D+F	57	18	31.6
Total material	132	24	

Class E not used.

Table III. *Urinary infection in relation to present and previous symptoms*

Urinary symptoms	No. of pats.	Patients with significant bacteriuria	
Present	12	9	75.0
Absent	120	15	12.5
Previously present	28	9	32.0
Previously absent	104	15	14 0

Urological symptoms

Table III shows the incidence of significant bacteriuria in relation to symptoms of disease of the urinary tract, either previous or present.

It is seen that 1/4 of the patients with symptoms of urinary tract infection did not have significant bacteriuria. It is even more remarkable that 62.5% (15 out of 24) of the patients with bacteriuria were asymptomatic at the time of investigation and throughout the entire course of pregnancy. Table III also shows that previous urinary infection is a poor index of current urinary infection. It was not possible to evaluate conditions around the time of delivery, as the patients received antibiotics prophylactically immediately prior to labour and many of them were catheterized. None of the patients were examined after delivery.

DISCUSSION

In previous studies (5, 7) a significantly greater incidence of significant bacteriuria was found among both selected and non-selected pregnant diabetics than in comparable control groups without diabetes mellitus. It was also shown previously that this increased incidence could be correlated with the angiopathy of long-term diabetes in non-pregnant women (6). This positive correlation also demonstrated in pregnant women in the study.

Pometta et al. (3) have examined a possible correlation of this nature in pregnant diabetics, but because of the restricted material they were only able to suggest that such a relationship might exist. The angiopathy may be considered a contributory factor in the development of urinary tract infection (6), but, as suggested by others (3, 4), the bacteriuria may possibly accentuate the angiopathy. It will be necessary to have controlled therapeutic studies to elucidate this further.

The relationship between bacteriuria and the diabetic state is emphasized by the finding in the present investigation of a significant correlation between bacteriuria and increasing duration of diabetes independent of chronological age. This may also be expressed by saying that patients with bacteriuria are mainly found in White's groups D and F.

The present and previous studies appear to add further emphasis to the relationship between angiopathy and bacteriuria and would seem to call for further pathophysiological studies of this question, including, among other things, the possible relationship between subclinical neuropathy of the bladder and significant bacteriuria.

ACKNOWLEDGEMENTS

This study was aided by grants from the Novo Foundation and the F. L. Smith's Foundation.

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Acta med. scand. Vol. 193 pp. 347–352, 1973

STUDIES ON URINARY INFECTIONS IN DIABETICS

V *Bacteriuria in Relation to Various Obstetrical Features, Foetal Outcome and Mortality*

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Abstract. On the basis of two consecutive urine cultures, in which the criterion for the presence of significant bacteriuria was $>10^5$ colonies/ml urine of the same micro-organisms, the relationships have been examined between bacteriuria and various obstetrical data in 132 pregnant diabetics. The findings were as follows. 1) There is no correlation between significant bacteriuria and the number of completed pregnancies and previous deliveries. 2) No relationship is found between complications during previous pregnancies, and significant bacteriuria during the current pregnancy. 3) Frequent cases of insulin shock were demonstrated in patients with significant bacteriuria, which occurred most frequently in White's groups D and F. Correspondingly severe acidosis was present in patients with bacteriuria more frequently than in patients without bacteriuria. 4) Out of 23 patients with proteinuria, 10 out of 19 (53%) also had significant bacteriuria. All these patients were in White's groups D and F. Of the patients in White's groups D and F 57% had at the same time significant bacteriuria and a pronounced rise of BP in connection with delivery. 5) Significant bacteriuria occurred much more frequently in patients delivered 10 weeks prior to estimated term than in other patients. However, the difference was not significant. 6) Out of 24 patients with significant bacteriuria, 10 went into spontaneous unexpected labour. In White's groups D and F the incidence of bacteriuria was 53% among those going into spontaneous, unexpected labour, compared to a figure of 23% among the other patients. The difference is not statistically significant. 7) Perinatal death appears to be more frequent among infants of mothers with significant bacteriuria. 8) The material included 19 cases of malformations. Only one out of 4 mothers with bacteriuria gave birth to an infant with malformations. 9) It is concluded that the problem associated with the question of bacteriuria in pregnant diabetics is still not elucidated, but is a significant one. As the number of variables is great, an international, multicentre investigation would appear desirable.

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The aim of the present study was to elucidate possible correlations between significant bacteriuria and a series of obstetrical data, as well as the significance of the bacteriuria for the course of labour and the perinatal state of the child of pregnant diabetics. These correlations have attracted considerable attention in the case of pregnant women in general and have occasioned contradictory opinions (3, 9, 13).

MATERIAL AND METHODS

The material consists of 132 non-selected diabetics who have been followed by means of repeated cultures of urine throughout pregnancy from the time of first attendance at the Diabetes Centre, on an average in the 26th week of pregnancy until delivery. Both the material and the procedures and definitions employed have been described in detail earlier (11, 12).

All dead infants were autopsied by I. Tygstrup, the University Hospital Institute of Infant Pathology.

Fisher's exact test was used in the statistical analyses.

Definitions (5, 6)

1) *Severe acidosis:* venous standard bicarbonate 10–17 mEq/l.

2) *Proteinuria:* >0.5% in three consecutive determinations.

3) *Blood pressure:* normal $>140/90$, slight elevation $>200/100$.

4) *Perinatal mortality:* stillbirth and neonatal deaths within 10 days after delivery of babies weighing 1 000 g and over.

5) *Premature birth:* weight less than 2 500 g.

6) *Congenital malformations:* the mildest type included is poly- and syndactyly. *Fatal malformations are those* which are sufficient to explain intrauterine death or which are incompatible with extrauterine life for more than 10 days.

7) *White's classification* (cf. 12).

Table I. *Significant bacteriuria in relation to number of pregnancies and parity*

	White group				
	A+B+C		D+F		
	Significant bacteriuria				
	+	−	+	−	Total
No. of pregnancies					
I	1	25	4	12	42
II	2	14	7	13	36
III	1	19	4	7	31
≥IV	2	11	3	7	23
Total no. of pats.	6	69	18	39	132
Parity					
I	1	30	8	19	58
	3	18	9	16	46
≥3	2	21	1	4	28

Table III. *Significant bacteriuria in relation to gestational age and to spontaneous premature delivery*

	White group				
	A+B+C		D+F		
	Significant bacteriuria				
	+	−	+	−	Total
Gestational age (weeks) <30	1	2	3	4	10
31–32	0	9	2	5	16
33–34	2	29	8	15	54
≥35	3	27	5	14	49
Total no. of pats.	6	67	18	38	129[a]
Spontaneous premature delivery	1	10	9	8	28

[a] Unknown in 3 patients.

RESULTS

Number of pregnancies and parity

Table I shows that the number of previous pregnancies and deliveries are without effect on the occurrence of significant bacteriuria.

Complications in previous and current pregnancy

From previous pregnancies a history of complicating factors (e.g. acidosis, toxaemia) which particularly affect diabetic women with angiopathy could not be correlated with significant bacteriuria during the current pregnancy. The conditions during the current pregnancy are set out in Table II. Of a total of 132 patients in the material 75 could be placed in White's groups A+B+C, 6 of whom had significant bacteriuria. Of the 57 patients in groups D+F 18 had bacteriuria. The Tables show the relationship between various clinical variables of pregnancy and delivery and the occurrence of bacteriuria as well as the position in White's classification.

Table II. *Significant bacteriuria in relation to some complications during the current pregnancy and in relation to BP during pregnancy and at partus*

	White group				
	A+B+C		D+F		
	Significant bacteriuria				
		−	+	−	Total
Total no. of pats.	6	69	18	39	132
Venefichum insulin	1	14	8	10	33
Severe acidosis	1	4	6	5	16
BP elevated in pregnancy	0	5	7	3	15
Proteinuria	0	4	10	9	23
Highly elevated at partus	0	5	8	4	17
Slightly elevated at partus	1	10	2	8	21

Insulin shock occurred in 33 patients, more than half of whom were in White's groups D and F. The incidence of bacteriuria in patients with insulin shock was 44% in groups D+F (8/18) and 7% in groups A+B+C (1/15) ($p > 0.05$).

Severe acidosis was found in a total of 16 patients, significant bacteriuria in 7 of them (44%); the incidences were 55% in groups D+F and 20% in groups A+B+C.

Twenty-three patients had proteinuria during pregnancy, 10 of whom had bacteriuria, all in groups D+F (55%). Correspondingly among patients with elevated BP during pregnancy there were none with bacteriuria in groups A+B+C, but 70% in group D (7/10).

Similar conditions were found when there was pronounced elevation of BP in connection with delivery. Eight of these 17 patients had bacteriuria, all in White's groups D+F (8/12), but none in groups A+B+C with 5 patients. It is worth noting that 3 patients from White's groups D+F who had bacteriuria, had both elevated BP and albuminuria during pregnancy, as well as

severe hypertension in connection with delivery The same groups without bacteriuria did not contain repeat cases.

Gestational age

A determination of gestational age is of particularly great significance in diabetic pregnancy with a view to the early induction of labour In the present study gestational age is calculated on the basis of the interval from the first day of last menstruation. Table III shows significant bacteriuria in relation to gestational age.

Significant bacteriuria occurs far more frequently in patients delivered 10 weeks before the calculated term than in other patients. Ten patients were delivered before the 30th week of pregnancy and 4 of them (40%) had bacteriuria compared with 20 (16%) in the remainder of the material. The difference is not significant ($p > 0.05$). This may also be expressed by saying that 4 out of 24 patients with bacteriuria (17%) were delivered before the 30th week compared to 6 out of 108 patients without bacteriuria (6%) ($p > 0.05$).

In 28 patients the onset of labour was unexpected and spontaneous before the calculated time for partus provocatus medicamentalis, 10 of whom (36%) had significant bacteriuria. In 56 of the patients in groups D+F 17 went into spontaneous premature labour and more than half of them had significant bacteriuria (9 17 53%), while only 9 out of 30 (23%) who underwent partus provocatus had bacteriuria. This difference is not statistically significant ($p > 0.05$)

It thus appears as if an existing bacteriuria might be followed by spontaneous and premature delivery but the material is too small to confirm this conclusion.

Table IV *Significant bacteriuria in relation to foetal outcome and to malformations*

	White group A+B+C		White group D+F		
	Significant bacteriuria				
	+	–	+	–	Total
Foetal outcome					
Liveborn	3	56	12	27	98
Asphyxia	2	8	1	7	18
Stillborn	0	3	2	5	10
Neonatal death	1	4	3	2	10
Total no. of pats.	6	71	18	41	136[a]
Malformations					
None	6	63	17	31	117
Slight	0	3	1	5	9
Fatal	0	5	0	5	10

T ins and triplets included.

Table V *Significant bacteriuria in relation to birth weight*

	White group A+B+C		White group D+F		
	Significant bacteriuria				
	+	–	+	–	Total
Foetal eight (g)					
<1 490–2 490	1	6	3	9	19
2 500–3 490	3	27	10	10	50
3 500–>4 500	2	35	5	18	60
Total no. of pats.	6	68	18	37	129[a]

Twins and triplets excluded.

Foetal outcome

The 132 patients gave birth to a total of 136 infants, including one set of triplets and two sets of twins. Table IV shows the condition of the infants at birth in relation to the presence of significant bacteriuria in the mother The same Table shows the relationship between congenital malformations and significant bacteriuria.

If this Table is analysed as a whole, without correction for multiple births, then 5 cases of malformation are found among the 10 stillbirths. None of these stillbirths were children of mothers with significant bacteriuria. In the case of the stillborn children of the mothers with bacteriuria, no explanation for these deaths was found at autopsy One of the mothers had pre-eclampsia

Among the 10 infants dying neonatally malformations were contributory in 5. The 4 infants who died neonatally and whose mothers had significant bacteriuria did not have malformations. The probable cause of death was asphyxia neonatorum in 1 respiratory insufficiency in 1 and respiratory distress syndrome in 2.

Perinatal deaths and malformations cannot be correlated with significant bacteriuria.

Birth weight

Table V shows the relationship between birth weight and significant bacteriuria. As expected, no association was found.

DISCUSSION

The present material of non-selected pregnant diabetic patients has been followed throughout the last period of pregnancy by means of repeated quantitative and qualitative urine cultures, and the patients have undergone a uniform control of their diabetic condition. In this group of patients it has been shown that significant bacteriuria occurs more frequently among pregnant diabetics than among pregnant non-diabetics (11). It has been shown that significant bacteriuria could be correlated with increasing duration of diabetes and vascular disease of late diabetes, evaluated by grading the retinopathy and as expressed by the White grouping. However it has not been demonstrated with certainty that significant bacteriuria is associated with diabetes mellitus per se (10, 12).

It has been shown in the present investigation that significant bacteriuria occurs far more frequently in patients who have proteinuria and elevated BP during pregnancy as well as in those who are delivered more than 10 weeks before the calculated term and who in connection with delivery have a pronounced rise of BP However these correlations only hold for women who have diabetic angiopathy The material is too small to decide whether there is a difference in the above mentioned variable in women with and without bacteriuria in groups D + F

It is worthy of note that significant bacteriuria could not be correlated with congenital malformations. The relation to perinatal death is doubtful. It should be emphasized once more that the material studied is limited so that no conclusions are justified.

The material was collected over a period of two years collection over a much longer period would involve other sources of error due to changed working procedures of an obstetrical and medical nature. To ensure a material which had undergone controlled treatment would have required a series of quite considerable dimensions (9 13)

The fact that it has not proved possible, as in so many other studies, to demonstrate a rise in the incidence of significant bacteriuria in relation to increasing number of pregnancies and deliveries may be due to the limited extent of the material and the low mean age, as well as to the infrequency of multiparity among diabetics in comparison with a mixed series.

The conditions associated with complications during previous pregnancies are difficult to evaluate in spite of a review of detailed records from the previous deliveries of the patients in question. It is remarkable that bacteriuria during the current pregnancy did not predominate in patients with previous toxaemia (1) There were 7 such patients, 2 of whom had significant bacteriuria. Nine patients had symptoms of urinary tract infection during previous pregnancies.

Examining the conditions in their present pregnancy where the patients with bacteriuria show a considerably greater tendency to acidosis and incidence of insulin shock than patients without bacteriuria, it must again be recalled that these are mainly patients with angiopathy

Ever since Peters et al. (7) in 1936 pointed out the significance of the observation, the relationship between toxaemia and urinary tract infection has been extensively discussed. Kincaid-Smith and Bullen (2) found that, whatever the age and parity significantly more women with bacteriuria develop elevated BP later in pregnancy even though they had normal BP on the occasion of the first pregnancy examination.

Examining 253 pregnant diabetics, Pometta et al. (8) found that the mean systolic BP was significantly higher in women with microangiopathy (i.e. > 1 microaneurism) than in patients without angiopathy They found no significant difference either in systolic or diastolic BP in patients with or without significant bacteriuria. The relationship found in the present study has no obvious explanation, but may be a consequence of existing diabetic angiopathy

The most controversial question in the complex of problems associated with bacteriuria and pregnancy has been the significance of bacteriuria for prematurity and perinatal mortality Kass (2) was the first to point out this relationship at the same time he demonstrated that antibacterial treatment considerably reduced the risk. A few investigators have since supported his view (2) although many others, in various attempts

(11) have been unable to show that bacteriuria disposes to prematurity

The corresponding circumstances in pregnant diabetics in particular were pre iously investigated by Pometta et al. (8). The incidence of significant bacteriuria among their 253 patients was 7% Fourteen per cent aborted spontaneously but there was no significant difference between the groups with or without angiopathy not even when combined with bacteriuria. The remaining patients were followed until after the 28th week. A minor number of patients were treated on the basis of their symptoms. Because of the special circumstance that diabetics are often delivered by primary caesarian section in the 36th week of gestation, the above study made no attempt to evaluate the incidence of prematurity On the other hand, when the mothers had significant bacteriuria and angiopathy the perinatal mortality was found to be 50% in contrast to 15% in mothers without bacteriuria but with angiopathy This result is not significant, however in view of their restricted material. In the group without bacteriuria or angiopathy the perinatal mortality was 11%

The criterion usually employed for prematurity i.e. a birth weight <2 500 g, cannot be employed in the case of children of diabetic mothers, whose birth weight *is considerably higher* on other grounds (4). In the present investigation it was found that mothers giving birth spontaneously prior to the calculated date are to be found in the group with bacteriuria. If one also takes into account the fact that some of the patients with significant bacteriuria have been treated, then in *spite of the restricted nature of the material the* strong impression cannot be a oided that the bac terluria may be contributory to the prematurity" in pregnant diabetics, especially those with angiopathy although this condition, particularly in the present category of patients, is multifactorial. A considerably larger material will be necessary be fore the problem of a relationship between bac teriuria and prematurity can be elucidated.

The number of infants dying, approximately 15% (20/136), does not differ essentially from the mortality found in the department in the case of children of diabetic mothers (4). However it is striking that without correction for multiple births, 30% of these mothers had significant bacteriuria, in contrast to the 15% of mothers with living infants. This relationship is likewise an interplay between a great number of factors, in particular in this case the degree of angiopathy

Previously only few investigators ha e compared their bacteriological findings with the presence of congenital malformations. Sa age et al (9) found that, of whatever nature, malformations were 30% more frequent among infants born of mothers without bacteriuria. In the present study congenital malformations appear to show no relationship to the occurrence of significant bacteriuria.

It must be emphasized that the cause of death in infants born of mothers with bacteriuria was respiratory insufficiency which corresponds to the findings of other investigators (8, 9).

To sum up it may be stated that significant bacteriuria occurs frequently among pregnant diabetics, and that this bacteriuria may result in further complications during the diabetic preg nancy

It is possible that the bacteriuria plays a minor part and is overshadowed by other known and unknown factors influencing the result, but until there is greater certainty as to the significance of the bacteriuria an examination for it should be made during pregnancy

The problem, however appears so significant, *and the number of variables so great, that one* strongly feels the need for an internationally directed major investigation with common lines of procedure.

ACKNOWLEDGEMENTS

This study was aided by grants from the Novo Foundation and the P L. Smith's Foundation

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Acta med. scand. Vol. 193 pp. 353–357 1973

CYTOGENETIC STUDIES IN HAEMATOLOGICAL DISORDERS WHICH MAY TERMINATE IN ACUTE LEUKAEMIA

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Abstract. Cytogenetic studies have been performed in 35 patients with potentially leukaemic haematological disorders. Abnormal stem lines were present in six patients, three of whom died from leukaemia or aggressive myelofibrosis within a few months. One patient developed leukaemia more than four years after the demonstration of the abnormal stem line. The chromatin of the abnormal bone marrow metaphases from these patients had a blurred appearance. The remaining two patients survived for 22 and 39 months without evidence of leukaemia. In these cases the chromatin structure of the marrow metaphases had a normal appearance. Three of the 29 cytogenetically normal patients developed leukaemia. It is concluded that the abnormal stem lines may be considered leukaemic clones of cells. However, a few patients may survive for months or years. The appearance of the chromatin structure of the bone marrow metaphases seems to be of prognostic significance. Finally evidence is given that the chromosome abnormalities are present in both cells from the granulocytic cell line and the erythroblasts of the bone marrow.

Cytogenetically abnormal clones—abnormal stem lines—are present in the bone marrow of about 50% of patients with acute leukaemia (5, 6, 14, 16). They are demonstrated at a very early stage of the illness and often occur prior to the full-blown blastic picture characteristic of acute myeloid leukaemia (5, 6). Abnormal stem lines are also present in a few patients suffering from haematological disorders which may terminate in acute leukaemia, i.e. polycythaemia vera, myelofibrosis, aplastic anaemia, sideroblastic anaemia, pancytopenia and aregeneratory anaemia (1, 3, 4, 10, 11, 12, 13, 15, 17). For some years we have studied patients with these disorders cytogenetically as such studies may help to elucidate the relation between abnormal chromosome complements and neoplasia and define the prognostic and diagnostic value of chromosome studies in haematology.

MATERIAL AND METHODS

The material consists of 35 patients, 15 males and 20 females, who have been followed from 19 to 75 months after the chromosome study. Their ages ranged from 21 to 82 years. Table I shows the types of haematological disorders studied. One of the patients had received X-irradiation of the spleen immediately prior to the chromosome study. None had been treated with radiophosphorus or cytostatics. In all the patients chromosome studies were performed on bone marrow material according to the methods described by Tjio and Whang (18) and Lam-Poo-Tang (9).

The proportion of bone marrow mitoses belonging to the erythrocytic and granulocytic precursors was determined in Giemsa-stained smears of the same aspirates as were used for cytogenetic studies. Mitoses were classified as erythroblasts if the mitotic cells corresponded in size and tinctorial characteristics to proerythroblasts, basophilic or polychromatic erythroblasts. All other mitoses were considered as non-erythroid. The differential count of mitotic figures was based on 100 mitoses in each patient.

RESULTS

Abnormal stem lines were present in 6 of the 35 patients. Table II depicts the chromosomal findings in these patients. In patient 1 about 95% of the metaphases contained 47 chromosomes. The hyperdiploidy was due to an extra chromosome in group C. The chromosomes belonging to the abnormal clone had a blurred appearance. In patient 2, 29 out of 50 metaphases had 48 chromosomes with two supernumerary chromosomes in group C. The chromatin had a normal appearance. The bone marrow of patient 3 was

Table I. *Types of haematological disorders studied*

Diagnosis	No. of pats.
Polycythaemia vera	7
Myelofibrosis	11
Idiopathic thrombocythaemia	2
Sideroblastic anaemia	3
Aplastic anaemia	5
Pancytopenia	6
Aregeneratory anaemia	1

studied twice at an interval of more than four years. Both aspirates had a hyperdiploid mode, the majority of the metaphases containing 49 chromosomes. There were three supernumerary chromosomes in group C. The metaphases of the first aspirate had normal chromatin (Fig. 1), whereas many metaphases in the second aspirate had a slightly blurred appearance. Patient 4 was studied twice. In the first marrow aspirate 5 out of 50 metaphases were abnormal. The metaphases contained 46 chromosomes with two acentric fragments slightly smaller than the chromosomes belonging to group G. In the marrow aspirate obtained five weeks later—18 days before death—22% of the metaphases had the same abnormal karyotype. The chromatin of the pseudodiploid metaphases had a very blurred appearance (Fig. 2). The bone marrow of patient 5 was studied three times. In the first aspirate about 25% of the metaphases were abnormal. They contained 44 chromosomes with a chromosome missing in groups C and D. In the second and third aspirates, obtained 2 and $3\,^1/_2$ months later, 60 and 80% of the metaphases had the same abnormal karyotype. The hypodiploid metaphases had blurred chromatin. In patient 6 all the metaphases studied were hypodiploid. The abnormal clone contained 45 chromosomes with a chromosome missing in group C. The chromatin had a normal appearance. In the remaining patients the chromosome complement was normal.

Two of six patients (nos. 1 and 4) with abnormal stem lines in the bone marrow developed acute myeloid leukaemia within three months after the chromosome study. One patient with myelofibrosis (no. 5) rapidly developed anaemia, thrombocytopenia and increasing numbers of myeloblasts in the bone marrow (15%)

Table II. *Chromosomal findings in bone marrow aspirates from six patients with abnormal stem lines in the bone marrow*

No.	Age (y)	Sex	Diagnosis	Date	Clinical course after the study	Total cells counted	Chromosome number <43	43	44	45	46	46*	47	48	49	>49
1	70	♂	Myelofibrosis	23.12.65	Dead from leukaemia after 3 mo.	19			1		2		16			
				27.1.66		50			1		2		47			
2	72	♀	Myelofibrosis	8.8.66	Dead from cardiac failure after 39 mo.	50					19		2	29		
3	33	♂	Pancytopenia	30.10.67	Leukaemia developed after approx. 50 mo.	50				2	3			2	43	
				4.4.72		50	1						3	8	37	1
4	68	♀	Myeloid metaplasia	30.1.69	Dead from leukaemia after 2 mo.	50	4	4	1	5	31	5				
				6.3.69		50	2		2	3	32	11				
5	79	♀	Myelofibrosis	8.7.69	Dead after $3\,^1/_2$ mo. Leukaemia?	22			5	1	16					
				8.9.69		32	1	3	15	1	12					
				17.10.69		50	3	2	33	2	8					
6	58	♂	Sideroblastic anaemia	13.5.70	Alv. after 22 mo.	50	2	1	6	41						

* Pseudodiploid metaphases.

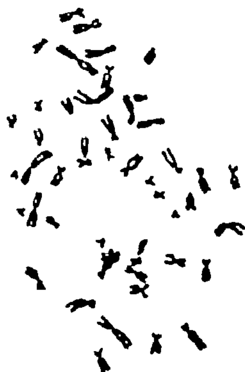


Fig. 1. Hyperdiploid metaphase from patient 3 with well defined chromatids and normal appearance of the chromatin.

and died within three months. Autopsy showed a hypoplastic bone marrow and extramedullary haemopoiesis in the spleen, liver and kidneys. The patient with pancytopenia (no. 3) is still alive, 53 months after the first chromosome study. However, at the beginning of 1972 his condition deteriorated and the bone marrow showed acute myeloid leukaemia. Another patient with myelofibrosis (no. 2) died at home from cardiac failure 39 months after the chromosome study; autopsy was not performed. The remaining patient with sideroblastic anaemia (no. 6) is still alive 22 months after the chromosome study. He shows no evidence of leukaemia.

Twelve of the 29 patients with normal karyotypes have died from one day to 60 months after the chromosome study. Three of them developed acute leukaemia. Sixteen patients are still alive.

In Table III the percentages of erythroid and non-erythroid mitoses in the marrow smears are compared to the percentages of normal diploid and abnormal aneuploid or pseudodiploid metaphases from the same aspirates. The counts show that at least in patients 1, 3, 5 and 6 the abnormal stem lines are made up of cells belonging to the erythrocytic as well as the granulocytic cell lines.

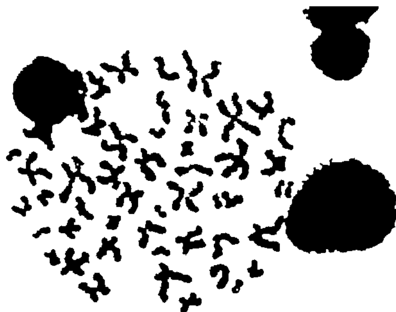


Fig. 2. Pseudodiploid metaphase from patient 4 with blurred appearance of the chromatin.

Table III. *Differential counts of bone marrow mitoses (%) compared to the proportion of normal and abnormal metaphases in four patients with abnormal stem lines in the bone marrow*

		Mitoses		Metaphases	
Pat. no.	Date	Erythroid	Non-erythroid	Normal	Abnormal
1	27 1.66	15	85	4	96
3	30.10.67	57	43	4	96
5	8.9.69	82	18	40	60
	17 10.69	44	56	20	80
6	11.5.70	61	39	0	100

DISCUSSION

Cytogenetic studies have obtained an increasing diagnostic and prognostic significance in patients with haematological disorders. However this raises the question whether the presence of abnormal stem lines in the bone marrow of patients with haematological diseases is synonymous with a leukaemic condition. The present series show that the demonstration of abnormal clones is associated with a high risk of developing leukaemia. However a few observations may suggest that the presence of abnormal stem lines does not always herald the development of leukaemia. Thus one patient of the present series [illegible] a few patients from the literature have [illegible]ed for years with an abnormal clone in [illegible] bone marrow without evidence of leukaemic transformation (2, 11 13). What is the interpretation of the abnormal clones in these patients who have not yet developed leukaemia? Nowell (11) has recently proposed that the patients who have survived for years with abnormal stem lines have no greater risk of developing leukaemia than the cytogenetically normal patients with the same disorders. However the number of patients reported with such "silent clones in the bone marrow is small and they have not been followed long enough to decide whether from a clinical point of view the abnormal stem lines are malignant or non-malignant. Previously we have considered the abnormal stem lines in these patients as representing leukaemic clones of cells which do not rapidly lead to a deleterious state, possibly due to a low "virulence of the cell line (4). That such a long-standing balance may exist between the organism and what is clearly a leukaemic cell line is well known from the clinical observations of smouldering leukaemia.

One finding indicates that there may be a difference between the nature of the abnormal clones in the two categories of patients, viz. patients who develop acute leukaemia within a few months after the demonstration of the abnormal stem line and patients who survive for years. Thus in the three patients of the present series who died within a few months from leukaemia and aggressive myelofibrosis, the chromosomes of the metaphases belonging to the abnormal stem lines had a blurred appearance as seen in leukaemic and other neoplastic metaphases. Contrary to this, the chromatin had a normal appearance in the two patients who have survived for 22 and 39 months with abnormal clones. In patient 3 the first bone marrow aspirate had chromatin of normal appearance. The chromatin had a blurred appearance in the bone marrow aspirate obtained 53 months later when acute leukaemia had developed.

The results of the present series together with the observations of Nowell (11) show that cytogenetic studies have a considerable prognostic value in patients with potentially leukaemic haematological disorders. We believe that an abnormal stem line is synonymous with a neoplastic clone of cells. Blurring of the chromatin in the aneuploid metaphases is a prognostically bad sign and actually means that a frank leukaemia is already present in the bone marrow A few patients, viz. those who have a chromatin structure of normal appearance, may survive for months or even years. In our opinion their condition may be considered leukaemic—although clinically not very malignant.

The data presented in Table III show that the chromosome abnormalities are not confined to either the erythrocytic or granulocytic cell line, but the metaphases from both cell lines make up the abnormal stem lines. This finding is in agreement with our and other authors previous findings in non-leukaemic haematological disorders (2, 4) and acute myeloid leukaemia (7 8). The involvement of the two cell lines, and possibly also the megakaryocytes, indicates that the alteration of the genome occurred in a stem cell common to the cell lines and may explain why abnormalities are often present both in the erythrocytic, granulocytic, and megakaryocytic series in

patients with polycythaemia vera, myelofibrosis, and aplastic anaemia. Therefore these diseases—like acute myeloid leukaemia—may be considered disorders of haemopoiesis in general.

ACKNOWLEDGEMENT

This work has been supported by a grant from Anders Hasselbalch's Fund against Leukaemia.

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Congress Announcement

The EPA Sponsoring International Congress on Noise will be held in Dubrovnik, Yugoslavia, May 13–18, 1973 The congress is being conducted by the Yugoslav Medical Association and the American Speech and Hearing Association. Attendance at the congress will be limited to 500.

Further information and applications to attend. The Office of Noise Abatement and Control, US Environmental Protection Agency Washington, D C. 20460, USA or The Yugoslav Medical Association Dr G Zarkovic, President, c/o The Institute of Hygiene and Social Medicine, University of Sarajevo, Sarajevo, Yugoslavia.

Acta med. scand. Vol. 193 pp. 359–362, 1973

DIABETES MELLITUS WITH OPTIC ATROPHY— THALASSEMIA LIKE SIDEROBLASTIC ANEMIA AND WEAK ISOAGGLUTININS— A NEW GENETIC SYNDROME?

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Abstract. This paper reports two cases: young man with Klinefelter's syndrome and his sister. Both siblings have diabetes mellitus, heredoataxia with optic atrophy thalassemia-like sideroblastic anemia, weak isoagglutinins and bone defects. A sister who died in early childhood suffered from an obscure anemia. The possibility of one or several genes influencing each other' penetrance is considered.

Juvenile diabetes mellitus combined with primary optic atrophy has been regarded as an expression of a specific genetic abnormality Rose et al. (29) in 1966 reviewed the literature and described seven cases. Since then additional reports ha e appeared (5, 12, 16, 24 28, 32). Neurogenic deafness has been considered as a third component in the syndrome (3 28). Other neurologic defects hav been described in that connection, as has diabetes insipidus. Sideroblastic anemia has only been mentioned in a few reports (6, 22). Therefore it might be of interest to report a man with Klinefelter's syndrome and his sister who both have juvenile diabetes mellitus, heredoataxia with optic atrophy thalassemia like sideroblastic anemia, bone malformations and weak isoagglutinins.

CASE REPORTS

Case 1

An 18-year-old man with vision difficulties since birth. At the age of 8 optic atrophy was diagnosed. At the same age he suffered skull fracture without complications. Anemia was diagnosed at the age of 1, which was treated with iron and vitamin B_{12} without effect. When he was 9 he showed walking difficulties. Investigation resulted in the diagnosis chondro-osteodystrophia Brailsford. Muscle biopsy from the quadriceps showed nothing pathological except small epithelioid cell granuloma.

At the age of 18 he presented classical juvenile diabetes mellitus, which was ell regulated with Insulin NPH, 40 IU in the morning and 4 IU in the afternoon, together with phenformin 50 mg twice day Intellectually he was normal to supernormal. He had pronounced dental caries, abolished tendon reflexes in the legs, reduced proprioceptibility in the feet and lowered vibration sense over the malleoli. Romberg's sign was positive. His right leg was 2 cm shorter and the thoracic kyphosis was marked, as was the lumbar lordosis, which was normalized in sitting position. His right ankle was anchylotic and his left foot showed reduced dorsal flexion. Both elbows showed 30° extension defects. Bilateral hallux algus was noted. The testes were atrophic. Ey examination showed an undulating nystagmus and optic atrophy bilaterally with 1–2/60 visual acuity The visual fields could not be determined. The audiogram was normal.

X-rays of the skeleton showed moderate frontal internal hyperostosis and thoracic kyphoscoliosis with edge-shaped vertebrae. Coxa plana ere noted in the hips. The epiphyseal margins were open distally in the radius and ulna bilaterally in all metacarpal bones except metacarpus II, in the proximal part of the humerus and distal part of the femur bilaterally and in both ends of the tibia. The second metacarpal bone in the left hand was shorter than the other and slightly deformed, and in the feet there was subluxation between thalus and tarsal scaphoid. The sphenoidal sinus was of normal size.

His blood group was A Rh (+) and the B-isoagglutinins ere reported to be very weak, being demonstrable only at +4°C. There was no weak B-antigen demonstrable in the erythrocytes. The saliva was not examined.

Hematologic examination showed: Hb 8.7 g%, RBC 2.8 mill. WBC 1 600–3 700 and thrombocytes 315 000–890 000/mm³. Reticulocytes were 27–101‰. Differential count showed 0.5–12.5% eosinophils, 18.5–32% monocytes, 15–42 nucleated erythrocytes/200 leukocytes, Jolly-bodies and basophilic stippling of the erythrocytes on several occasions. The bone marrow contained increased amounts of reticular iron, at least grade 4 according to scale 0–4, with 63% ring sideroblasts and many siderocytes.

MCV was 90–100 μ^3 and MCHC 30%. Serum iron was normal to increased; vitamin B_{12} and folic acid were normal, and so was the haptoglobin level. Hemoglobin electrophoresis showed normal amounts of HbA_2 and a slight increase of HbF to 4% (normal 0–1.8%). Glucose-6-phosphate dehydrogenase was normal.

Chromium-labeled erythrocytes disappeared with a rapidity of 4.9%/day (normal < 2.5%). Ferrokinetic studies showed low incorporation of Fe^{+} in RBC, high plasma iron turnover and the desferrioxamine test revealed high rates of iron excretion in the urine. Amino acid chromatography of urine was normal. Mucopolysaccarides were excreted in urine at three times the normal values (63% heparitin sulphate, 36% chondroitin sulphate).

Chromosome analyses from skin specimens showed, in all examined cells, an extra chromosome belonging to the group 6-X L. In buccal smear sex chromatin was demonstrated in 20% of the cells, which supports the diagnosis of Klinefelter syndrome.

Case 2

A 23-year-old sister of the first patient has had congenital luxation of the hips and reduced vision since early childhood. At the age of 8 bilateral optic atrophy was diagnosed. She has had anemia since childhood, treated on one occasion with iron without effect. On account of her brother' disease she as called for examination, but prior to this she developed classical juvenile diabetes mellitus and was treated in another hospital. She was later reexamined by the author. Her diabetes was well regulated with Insulin Novolente, 24 IU day

Intellectually she was normal to supernormal. She had moderate dental caries, areflexia in the legs and reduced vibration sense over the malleoli and positive Romberg sign. There was an extension defect in the elbows 20° with excessive rotation ability in the hips. Typical ing gait and bilateral hallux algus were noted.

Eye examination revealed nystagmus, bilateral optic atrophy and vision acuity of 3/60. The outer limits of the vision fields ere normal and no central scotomas could be demonstrated.

X-rays showed luxation of the hip joints and hallux algus. On the right side the renal pelvis and ureter were double.

She belonged to blood group A Rh (+) and the B-isoagglutinins ere very eak, being demonstrable only at +4°C. No weak B-antigen could be detected in the erythrocytes. The saliva was not examined.

Hematologic examination showed Hb 7.8 g% RBC 2.7 mill, WBC 2 700 and thrombocytes 300 000–870 000 mm³ Reticulocytes were 34‰. Differential count showed 5.5% eosinophils, 18% monocytes, 132 nucleated erythrocytes/200 leukocytes, Jolly-bodies and basophilic stippling of erythrocytes. The bone marrow contained an increased amount of reticular iron, at least grade 4 according to scale 0–4 with 66% ring sideroblasts and some siderocytes.

Serum iron was normal and so were vitamin B_{12}, folic acid and haptoglobin. Hemoglobin electrophoresis showed normal amounts of HbA_2 and 4% HbF (normal 0–1.8%). 3.4% of chromium-labeled erythrocytes disappeared per day (normal < 2.5%). Ferrokinetic studies gave similar results to those of her brother. Liver biopsy showed hemosiderosis.

Chromosome analysis of 38 cells from her skin showed normal conditions in 36—two cells appeared to have an extra chromosome belonging to group 17–18. However this might have been due to an artifact.

Heredity

Two children of the mother first cousin, but no other relatives, have diabetes mellitus. The parents were not consanguinous. The mother was hospitalized for pulmonary tuberculosis and died at 50 years of age, possibly from myocardial infarction. No autopsy was performed. The 64-year-old father has had duodenal ulcer and belongs to blood group O Rh (+). He has normal hematologic status and hemoglobin electrophoresis. One brother of the two patients was stillborn. Another sister died at 8 years of age from croup.

An older sister was examined when she was 5 for gait difficulties. Extension defects of 30° were noted in the elbows, and of 15 in the knees. The ankles were supinated bilaterally and the capsules of the wrists and some interphalangeal joints were swollen.

Hematologic examination showed: Hb 54–70%, RBC 3.5–4.5 mill, WBC 2 900–6 200 and thrombocytes 275 000–430 000 mm³ Differential count showed 55% neutrophil granulocytes, 1% eosinophils, 9% monocytes, 28% lymphocytes, 3% myelocytes, 3% metamyelocytes, and 3% myeloblasts.

Gastric secretion test after test meal showed achlorhydria. Treatment with hydrochloric acid and iron, however had no effect on the anemia. A diagnosis of SAF disease was made. She died at the age of 6, reportedly from heart disease. No autopsy was performed.

DISCUSSION

Both siblings had verified optic atrophy before the manifestation of diabetes mellitus. The optic atrophy is not of the type seen in Leber's disease. The brother had a normal audiogram and his sister's hearing ability was normal on clinical examination. The other neurologic symptoms noted are not quite of the type seen in Friedreich's disease. The patients normal intelligence and areflexia in the legs do not fit with Behr's syndrome. Their neurologic picture is more like the one described by Lundberg et al. (21) in a family of fifteen members in which diabetes mellitus was noted only in a 19-year-old man, who had also slight atrophy of his testes. There are many forms of heredoataxia and they do not always seem to be distinctly separated from each other The frequency of diabetes mellitus is best investigated in Friedreich's ataxia. Thorén (33) found manifest diabetes in 18% and Hewer and

Robinson (15) in 8% which is considerably more than in the normal population. Friedreich's ataxia is inherited in a recessive autosomal way and, when combined with diabetes, it has been postulated that the gene of Friedreich's disease affects the penetrance of the diabetes gene so that even heterozygotes develop manifest diabetes mellitus (28).

In cases with optic atrophy and diabetes mellitus, Ikkos et al. (16) found no sex difference, but when diabetes insipidus was also present they found a pronounced female predominance. Among patients with this syndrome there was one man among seven women, but Francois (12) as also Bretz et al. (5), described cases in two brothers, so the sex difference is no longer so impressive.

Ring sideroblasts occur in sideroachrestic anemia, which, in its hereditary form, is found almost exclusively among men and is considered to be X-bound or sex-influenced (7 11 20, 22, 27 30). In thalassemia there are also ring sideroblasts in the bone marrow but the peripheral blood contains many nucleated erythrocytes, which is not the case in sideroachrestic anemia. There is also an increased amount of HbF The two siblings described in this report have a clear cut sideroblastic anemia and, considering the peripheral blood and the hemoglobin electrophoresis, the hematologic picture fits with thalassemia. There was no indication of splenic aplasia which could explain the presence of the Jolly-bodies. One sister also had anemia, but its type was never established. Only one case of thalassemia in a patient with Klinefelter's syndrome has been found in the literature (9) and was regarded as a mere coincidence. Sideroblastic anemia together with optic atrophy and diabetes mellitus has been described twice (6, 22). In sideroachrestic anemia, as in thalassemia, there is an increased iron deposit in the parenchymatous organs and diabetes mellitus is not uncommon. The reason for that might be hemochromatosis, which is common in these diseases (14) but occurs also in hemosiderosis (1) Nevertheless one must consider the possibility that this is a disease entity in which one or more genes influence each other's penetrance.

Both siblings have weak B-isoagglutinins, detectable only at +4 C. That defect can also be genetically determined (10, 19).

The male patient was diagnosed as having Brailford's disease, which, however does not fit with the X-ray examination. He also excreted large amounts of urinary mucopolysaccharides in a form which could point to a mild form of Sanfilippo's syndrome, but this is not in accordance with the clinical picture. The sister also had bone defects, as did the deceased sister with obscure anemia. Various bone defects occur in Klinefelter's syndrome, and the epiphyseal margins close late in that disease (4 31), but that is also true in thalassemia (2).

Whether the Klinefelter's syndrome has anything to do with the neurologic disturbances is difficult to say with certainty but it has been reported in association with cerebellar heredoataxia (17). One man with diabetes mellitus, Friedreich's ataxia with optic atrophy and hypogonadism has also been described (8), so such a connection might be possible.

Diabetes mellitus has also been reported more frequently in Klinefelter's syndrome (25 26 34), even if this has not been verified in a few studies (18 23). It cannot be excluded that diabetes mellitus in the patient with Klinefelter's syndrome is linked to the chromosomal aberration. Since the sister also has diabetes, and they both have optic atrophy it seems more likely that some common genetic factor is responsible. Whether it is a pleiotropic manifestation of the same abnormal gene, according to Fraser (13), or several genes which influence each other's penetrance cannot be determined from these two cases. Further reports about similar patients will perhaps shed more light on this problem

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ANERYTHRAEMIC DI GUGLIELMO SYNDROME

Report of a Case

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Abstract. As there may perhaps be tendency to disregard diagnosis of acute erythraemic myelosis when there are no erythroblasts in the peripheral blood, such case observed in 69-year-old man with pancytopenia and hypercellular bone marrow is reported. Of the bone marrow cells 85% are erythroblasts, most of which were basophilic. Many of the cells were megaloblastoid, often multilobular or multinuclear with strongly positive PAS reaction. No erythroblasts were observed in the peripheral blood. The case must be classified as one of anerythraemic, aleukaemic Di Guglielmo syndrome.

Various types of leukaemia may occur in both a leukaemic and an aleukaemic form. However in the literature, one of the most frequently cited diagnostic criteria (5 7 8) for the Di Guglielmo syndrome, i.e. acute erythraemic myelosis, is that the peripheral blood contains erythroblasts, which in pure erythraemia must be interpreted as the leukaemic cells. Thus this concept of the disease comprises only a leukaemic type. Accordingly there seems to be a tendency to disregard a diagnosis of acute erythraemic myelosis when no erythroblasts are present in the blood. For this reason we found that it would be of interest to report a case which must be classified as an anerythraemic form of this type of leukaemia.

CASE REPORT

In June 1970 man, aged 69 years, was admitted to local hospital with 2-month history of increasing fatigue. The Hb level was 6 g/100 ml, WBC 2 800/µl with normal distribution, platelets 190 000/µl. The patient was treated with blood transfusions and then transferred to our hospital.

Apart from signs of anaemia and some bleedings into the skin, the clinical examination did not reveal any abnormalities. The Hb level was 6–7 g 100 ml, WBC 1 900–4 100/µl with preponderance of neutrophils with segmented nuclei, few myelocytes and juveniles but no myeloblasts were observed. The red blood picture showed anisocytosis, but otherwise no abnormalities. Reticulocytes were absent. The platelet count was now reduced to 40 000/µl. The bone marrow was markedly hyperplastic (Fig. 1). Of the cells 85% were erythroblasts, most of which were pathological. The majority of the pathological cells were pro-erythroblasts and basophilic erythroblasts, often multilobular or multinuclear (2–5 nuclei). The cytoplasm was strongly basophilic, often vacuolated or with an irregular cell border with conspicuous budding and fragmentation (Figs 2 5). The more mature erythroblasts revealed distinct megaloblastoid changes with abundant amounts of cytoplasm and often some constricted-off portions of nuclear chromatin or nuclear lobuli, up to 10–12 lobuli were occasionally observed. In addition, the number of myeloblasts as slightly increased. Many of the erythroblasts showed strongly positive PAS reaction (Fig. 6). The granulopoiesis did not reveal any megaloblastoid changes. The level of serum iron was considerably increased, while serum transferrin was within the normal range. Serum haptoglobin as reduced on one occasion and normal on another The serum levels of folic acid and vitamin B_{12} are normal Studies of gastric secretion revealed histamine-fast achlorhydria. The Schilling test showed normal conditions. The FIGlu test was markedly pathological with 143 mol/h of excreted urinary FIGlu after histidine load (normal level less than 20 mol/h). The direct Coombs test was negative.

Combination chemotherapy with daunorubicine and cytosine-arabinoside failed to give any remission. The patient died 10 days after the institution of therapy

Autopsy was performed (Dr F Lass). Histological examination revealed massive hyperplasia of the bone marrow with infiltration by the previously described pathological cells. The normal structure of the spleen was blurred; white pulp was practically absent, and the red pulp was transformed into bone marrow-like tissue with violent proliferation of the same cell type. Accumulations of these cells are also observed in the sinusoids of the liver and in the lymph nodes. Examination of the kidney showed

perirenal haemorrhages with many abnormal erythroblasts, which were also present in the interstitial spaces of the renal parenchyma.

DISCUSSION

The bone marrow hyperplasia with a greatly increased number of pathological erythroblasts seems to be compatible only with a diagnosis of acute erythraemic myelosis. The cell morphology is in complete agreement with previous descriptions of the pathological erythroblasts seen in this syndrome (2, 5)

Vitamin B_{12} or folic acid deficiency should particularly be considered in the differential diagnosis (1 4) but the serum levels of both these vitamins were normal. The patient had achlorhydria, but the Schilling test was normal. A pathological FIGlu test does not seem to be specific for folic acid deficiency (3 8) The presence of megaloblastoid changes in the erythroblasts without a similar maturation disturbance in the granulopoiesis is evidence in support of the diagnosis of erythraemic myelosis (8) The same applies to the strongly positive PAS reaction of the erythroblasts (5 6). The presence of coarse granules in the PAS reaction indicates that the erythroblasts are highly immature (5). The histological studies revealed infiltrations by pathological cells in the bone marrow spleen, sinusoids of the liver and lymph nodes and in the kidneys, which confirms the diagnosis of leukaemia.

Numerous examinations of blood smears did not reveal any erythroblasts, which is in agreement with the fact that reticulocytes were also absent. In acute erythraemia with erythroblasts in the blood the reticulocyte counts are often increased (1)

The case reported above may thus be taken as evidence that aleukaemic cases of this acute leukaemia also occur In his original report, Di Guglielmo gave the following definition of the disease: Les maladies érythrémiques sont des entités pathologiques autonomes, c est-à-dire des maladies véritables et spécifiques, primitives, caractérisées par une prolifération systématique et généralisée, qui touche électivement l'appareil érythropoietique du tissu myéloide. Just after this definition he gave his classification of the various types of the disease: la myélose érythrémique à forme néoplasique and la myélose anérythrémique

The literature seems to contain only a few subsequent descriptions of the Di Guglielmo syndrome without erythroblasts in the blood (1 6). Kawakita (6) uses the term anerythraemic for cases without erythroblasts in the blood, while by aleukaemic he understands a condition without myeloblasts in the blood. According to this terminology our case must be classified as both anerythraemic and aleukaemic.

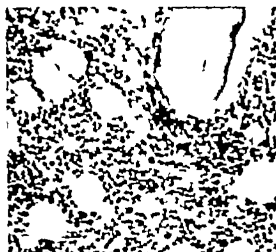
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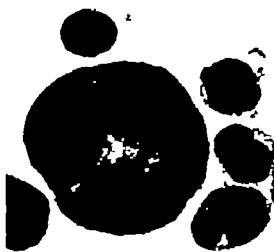
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Fig. 1 Crista biopsy showing hypercellularity of the bone marrow 160.

Figs. 2 5 Pathological erythroblasts of the Di Guglielmo type. 1 000.

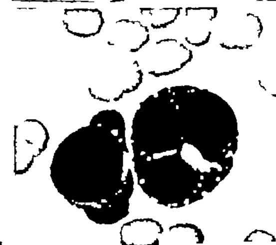
Fig 6. Basophilic ry hroblasts ith strongly positive PAS reaction 1 000.


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A CASE OF SYNCOPE ON SWALLOWING SECONDARY TO DIFFUSE OESOPHAGEAL SPASM

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Abstract. A case of secondary vagal syncope elicited from the oesophagus is reported. Radiography and manometry revealed changes characteristic of diffuse oesophageal spasm. The intake of granulate, which in the patient's experience could induce the attacks in mild form, was found to elicit precordial pain, an abnormally high intraluminal pressure in the oesophagus, and atrio-ventricular block. After being treated by oesophagomyotomy plus repair of hiatal hernia the patient has not for 6 months had any pain from syncopal attacks.

It is an established fact that organic diseases of the oesophagus may by way of reflex, elicit cardiac arrhythmia and syncope. In rare cases the same *reflex may* be elicited by a *functional* disease, oesophageal spasm. In those cases the diagnostic proof of a causal relationship can be difficult to obtain and we shall therefore report our experience of a single case, laying particular emphasis on the value of the manometric investigation.

CASE REPORT

A woman, aged 64, was admitted in 1966 as an emergency to the medical departments with precordial pain. The pain had arisen suddenly while the patient was at rest and it subsided shortly after admission. She had never previously had *similar episodes, and as no signs* of cardiac disease could be found, she was discharged without treatment.

In 1968 she was admitted to the Department of Neurosurgery with signs of low cervical slipped disc which, however, could not be demonstrated with certainty but the sensations in the left arm were persistent.

In Jan. 1971 she was admitted to the Department of Thoracic Surgery when radiography of the oesophagus and stomach had disclosed hiatal hernia of the sliding type. During the last few years she had suffered repeated attacks of pain of the same type as on the occasion of her first admission to the hospital. During the preceding year the pain had often been accompanied by unconsciousness lasting for 5 or 6 sec, and gradually the episodes showed special sequence: first slowly increasing pain, thereafter short-lasting syncope, and when she regained consciousness the pain had disappeared. The frequency of these episodes was extremely varied. As rule there was an interval of several days, but sometimes 3 or 4 attacks occurred on the same day and in one of the first attacks she had sustained slight injury by falling.

During the same period she had slight dysphagia and feeling of regurgitation, but never in connection with the intake of fluids or solids.

During short period she had received granulated agent, Mocol® for mild constipation and she had noted that this agent could elicit mild attacks. The pain was not so intense, and she did not actually lose consciousness, but had brief sensation of weakness and dizziness.

Cineradiography of the oesophagus revealed, in addition to the hernia, tertiary contractions in the lower part of the oesophagus with stagnation and regurgitation of the barium.

Investigations were made of the effect of swallowing the granulated agent upon the ECG and the intraluminal pressure in the oesophagus. The manometric study was performed with thin polyethylene catheters with constant flow (6). The pressure was recorded by transducers (Elema-Schoenander, EMT 35), and the pressure curves were traced on Mingograph 81 (Elema-Schoenander).

Fig. 1 shows the radiographic findings and the manometric tracing of dry swallow. The motility of the oesophagus *is abnormal,* as *contractions* occur simultaneously at various levels, and the after-contraction of the sphincter is prolonged (normally less than 12 sec).

Swallowing after the intake of the granulate elicited typical changes of the spastic oesophagus (Fig. 2). The contractions occurred simultaneously were repetitive and of high amplitude.

Registration of an ECG (V_1) simultaneously with the swallowing of granulate showed in few seconds bradycardia and prolonged conduction with transition into shortlasting atrio-ventricular block (Fig. 3). During the subsequent 2 min these abnormalities occurred several times, the patient complaining of pain and dizziness.

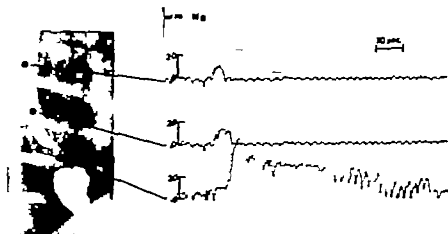


Fig. 1. Pressure curves from the oesophagus in connection with a dry swallow. The mutual distance between the catheter openings is 5 cm, and the lower catheter is in the displaced sphincter, abov the radiologically uible hiatal hernia.

Thereafter she was symptom-free and the ECG was normalized.

It was striking that the first ECG changes occurred ery early in the act of swallowing. T ascertain whether a carotid sinus reflex was contributory and to elucidate in more detail the haemodynamic conditions in connection with the syncopes, catheters were inserted into the femoral artery and into the right atrium from the femoral vein. Simultaneously an ECG (lead I) and the pressure in the distal, spastic part of the oesophagus were recorded. Massage of the right carotid sinus induced only trace of bradycardia, whereas pressure on the left carotid sinus immediately elicited atrio-ventricular block with declining pressure and increasing atrial pressure (Fig. 4). During massage of the carotid sinus the oesophageal pressure remained normal and stable.

Fig. presents recording of ECG and pressure changes in the right atrium, femoral artery and oesophagus during the intake of granulate. The patient was in the erect position which allegedly aggravated symptoms. An increase in oesophageal pressure was followed subsequently by bradycardia, prolonged conduction and atrioventricular block. At the same time decrease of arterial blood pressure and an increase of central enous pressure were seen. A yncope was not elicited, but the patient complained of pain and dizziness.

After the diseased area had been localized by manometry 10 cm long oesophagomyotomy and an Allison repair of the hernia were done. The oesophagus proved grossly normal. The postoperative course was uneventful, and the patient has not had pain or syncope after the operation. She has no dysphagia, but still mild reflux complaints which are controlled by medication.

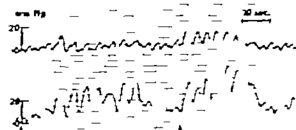


Fig. –. Pressure curves from the oesophagus during the swallowing of granulate. Each swallow is indicated by an arrow. The distance between the catheter openings is 5 cm, and the bottom curve reflects the highly spastic area just above the sphincter.

DISCUSSION

The combination of ECG, central venous pressure, arterial pressure and oesophageal pressure *appears to show that the granulate-induced oe*sophageal spasm elicits a agal reflex consisting of bradycardia and transitory atrio-ventricular dissociation. The resulting decrease in arterial pressure was in certain situations sufficient to cause unconsciousness and thereby the fullblown vagal syncope. The slightly rising pressure in the right atrium during the attack showed the unaltered venous flow and could indicate that the reflex does not include any major vascular component. This is apparent also from the finding that it was only in the erect position that the fall of blood pressure was sufficient to threaten the cerebral circulation. Muscular movements in the cervical *area in connection with the act of swallowing* may affect the extremely pressure-sensitive left

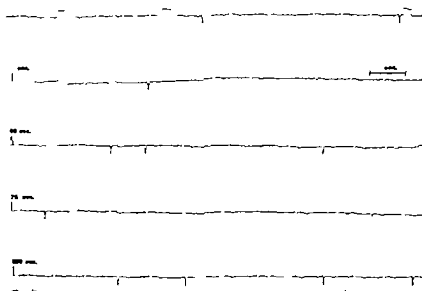


Fig. 3. ECG (V) traced before and after the intake of granulate. At the top the patient normal ECG. The other tracings exhibit the changes at various times after the patient had swallowed granulate and the normalization at the end of 2 min.

carotid sinus and be responsible for the early period of bradycardia. The subsequent attacks must have been elicited from the oesophagus, since they coincided with the spasms in this organ and *since at that juncture the patient did not* make any muscular movements. The result of the treatment also shows that the reflex elicited from the oesophagus was responsible for the symptoms.

The efferent nerve of the reflex must be the right and left vagus, *but whether the afferent* nerve from the oesophagus is the vagus or sympathetic trunk cannot be decided on the basis of this or other reported cases.

The eliciting factor an abnormally high tension in the oesophageal wall, may be produced by various mechanisms and the literature contains examples of syncope caused by organic as well as *functional diseases. Tolman and Ashworth* (8) Weiss and Ferris (9), Pedersen et al. (5) and Iglauer and Schwartz (2) have described such episodes in connection with peptic stricture, a diverticulum, and achalasia. The increase in tension *is secondary to an intraluminal pressure in*crement caused by alimentary stagnation and the causal relationship is obvious.

In two patients described by Corell and Lindert

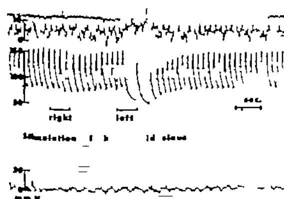


Fig. 4. ECG (lead I) and pressure curves from the right atrium, femoral artery and distal part of the oesophagus. Carotid massage clearly demonstrates the sensitiv left carotid sinus.



Fig. 5. ECG (lead I) and pressure curves from the right atrium, femoral artery and distal part of the oesophagus after intake of granulate. The pressure increment in the oesophagus is followed by atrio-ventricular block with drop of arterial BP and rising central venous pressure.

(1) and by James (3), respectively, an oesophageal diverticulum was also demonstrated, but in both cases the abnormal syncope appears to have been potentiated by digitalis medication.

In cases based on a purely functional disease the spastic oesophagus is reported by Kopald et al. (4) and Sapru et al. (7) but in the last mentioned investigation the diagnostic test did not include manometry. The true cause of the syncopes is not evident when dealing with functional diseases. However, a thorough unravelling of the individual symptoms and their time relations will arouse a suspicion of a secondary cause. Pain is one of the main symptoms of diffuse oesophageal spasm. It may be in the same site and of the same nature and intensity as in the ischaemic heart diseases, but it occurs spontaneously without relation to physical exertion. It may be provoked by the intake of certain foods or fluids such as carbonated beverages or, as in the case of Sapru et al. (7) by almost any form of food intake. In our case the long-standing and severe attacks were spontaneous, unrelated to the intake of fluids or solids, and the intake of Mucol® granulate elicited only short-lasting and mild attacks. Presumably the granulate, by reason of its consistency, has caused mechanical irritation of the mucosa and thereby elicited the spastic contractions.

Intermittent dysphagia of varying intensity is a common sign, but the patients usually neglect symptom and concentrate on the pain.

Cineradiographic examination should be the initial diagnostic procedure showing tertiary contractions and segmentation of the barium with stagnation and regurgitation. The changes are most marked distally and may or may not be associated with hiatal hernia.

The decisive diagnostic investigation is simultaneous electrocardiography and measurement of the intraluminal pressure in the oesophagus. The characteristic manometric appearance is forcefully repeated contractions occurring simultaneously at different levels upon each swallow, and the cardiac arrhythmia must coincide with the periods of high intraluminal pressure.

We should mention that both our and Kopald et al.'s patient had marked degenerative diseases in the lower cervical spine, but we are at the moment not sure that there is any relationship between spastic oesophagus and disorders of the cervical spine.

In cases where the diffuse oesophageal spasm is accompanied by serious reflex vagal syncopes, surgical treatment may be indicated. The case of Kopald et al. was treated by oesophagomyotomy and high bilateral vagotomy, which resulted in a considerable reduction of the frequency and intensity of the attacks. After division of the vagal as well as the sympathetic branches to the abnormal segment of the oesophagus, Sapru et al.'s patient was completely relieved of syncopal attacks, but developed mild diarrhoea. The present case was treated by myotomy plus repair of the hernia, and the patient has not thereafter had any pain or syncopal attacks. Of her preoperative complaints only a mild reflux persists, and this is being controlled by medication.

ACKNOWLEDGEMENT

This work was aided by a grant from the Danish Heart Foundation.

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Acta med. scand. Vol. 193 pp. 369–372, 1973

EDITORIAL

PLASMA LIPOPROTEINS AND HYPERLIPOPROTEINEMIA

The earlier classification of hyperlipidemias was based on the presence or absence of elevated plasma cholesterol and/or triglyceride values. It is now well recognized that these lipids, cholesterol and triglycerides, are transported in the form of so-called lipoprotein particles. These lipoproteins are characterized by different lipid-carrying proteins (so-called apo-lipoproteins A, B and C) (1), each with different mobility on electrophoresis due to their electrical charge. The lipid part, on the other hand, decides the specific weight—the density—of the lipoproteins and gives them different speeds of flotation in the ultracentrifuge. Like cream in the milk centrifuge, the chylomicrons first float as they have the lowest density. Variations in the relative lipid composition cause the differences in density between lipoproteins, as can be demonstrated in the lipoprotein spectrum (Fig. 1).

In addition to the major lipoproteins, other lipoproteins may occur in plasma. The Lp(a) lipoprotein disclosed by Berg (2) is a genetic variant of β-LP present in the density class 1.050–1.080. The phenotype Lp(a+) is found with a frequency of about 35% in population samples of healthy Caucasians. The Lp(a) lipoprotein appears to be synonymous with "sinking pre-β-lipoprotein" (15) and may be closely related also to the pre-β-1-lipoprotein (also referred to as "pre β-HDL) studied by Dahlén et al. (4). One of us (A. G.) has recently produced evidence indicating that a minor polypeptide component, the thin line peptide (11–13) residing preferentially in the α-LP part of the LP spectrum, is an apo-LP in its own right forming a fourth normally occurring plasma lipoprotein LP D (10). Lipoprotein X (LP X) is an abnormal low density lipoprotein found in several conditions with biliary obstruction. LP X is the carrier of the increased amounts of free cholesterol and phospholipid characteristic of these conditions. Recently it has been demonstrated that LP X is present also in patients with familial lecithin-cholesterol acyltransferase (LCAT) deficiency (16).

The fact that the plasma triglycerides are transported both with chylomicrons and pre β-LP and the plasma cholesterol with pre β-LP, β-LP and α-LP makes it desirable in conditions with elevated plasma lipids to be able to evaluate which lipoprotein is involved. In line with this the lipoprotein typing system was introduced (6). It is possible that this classification may not be final, but it has a value for present-day discussions even if an individual patient seems to be an exception.

Hyperlipoproteinemia types II A, II B and IV are those most frequently found. In a male population sample it may be expected that lipoprotein patterns indicating hyperlipoproteinemia will be revealed in altogether 15–20% of the subjects. In a sample of middle-aged males who have had myocardial infarction, hyperlipoproteinemia of types II A, II B or IV may occur in 50–60% of the individuals. Hyperlipoproteinemia types III and V are not so frequently found and type I is rare. In recent studies (4) it has also been suggested that "pre β-1 LP" occurs frequently in series of males who have had a myocardial infarction.

A frequent matter of dispute has been the question of how to draw the line between "normal" and elevated plasma lipid and lipoprotein levels. The relationship between plasma lipids and the occurrence of coronary heart disease (CHD) does not start at a certain borderline of plasma lipids, but rather the risk of CHD will gradually increase with increasing level of plasma lipids. For practical purposes, however, certain

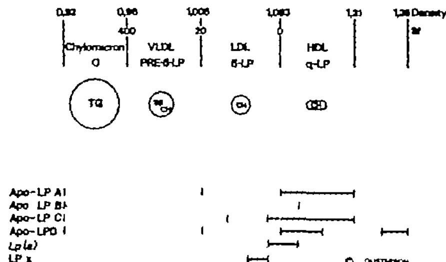


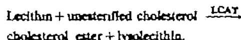
Fig. 1. Lipoprotein spectrum showing the two lipoprotein terminologies and density intervals and major flotation rates (J values) of lipoprotein classes. Applied to the spectrum are also the distribution of the apolipoproteins (apo-LP) and the lipoproteins Lp(a) and LP X. CH = cholesterol, TG = triglycerides.

limits have to be chosen. Fredrickson et al. (7, 8), based on their own experience, have suggested an upper normal value for β-LP cholesterol of 190–210 mg/100 ml, and for pre-β-LP cholesterol 35–40 mg/100 ml, in the age interval 40–60 years. The latter value corresponds roughly to a value of 180–200 mg/100 ml of plasma triglycerides. The plasma cholesterol value, on the other hand, is dependent not only on β-LP cholesterol but also on pre-β-LP cholesterol and α-LP cholesterol values. Considering 60 mg/100 ml as mean value for α-LP cholesterol, the upper level of total cholesterol in plasma would be about 280–320 mg/100 ml. These values, however, would not be looked upon as ideal lipid and lipoprotein values, but rather as borderlines acceptable for screening purposes.

For the time being the determination of plasma lipids, i.e. plasma cholesterol and triglycerides, should be adequate for the screening of presumptive cases of hyperlipoproteinemia, and also for the evaluation of the response to a lipid-lowering treatment. More elaborate techniques for lipoprotein typing may particularly be needed in cases with borderline plasma lipoprotein values and in cases with a combined elevation of plasma cholesterol and triglycerides. In these cases the lipoprotein typing should be performed with the patient in a metabolic steady state.

Lipoprotein electrophoresis does not permit quantitative studies of the lipoprotein distribution unless an additional, elaborate quantitation procedure is applied (5). Lipoprotein electrophoresis, however, if properly run, can suggest from the characteristic appearance of the lipoprotein bands the presence of hyperlipoproteinemia, types II B, III and V. Furthermore, lipoprotein electrophoresis can reveal the presence of chylomicrons as a cause of elevated triglyceride values in plasma from improperly fasted individuals.

The plasma lipoproteins transport plasma lipids, plasma triglycerides from intestinal sources in chylomicrons, and of preferentially hepatic origin in pre-β-LP. The free fatty acids (FFA) released by the hydrolysis of these triglycerides serve as an energy source for several functions in the body. The biological significance of having 2–4 g of lipoprotein-bound cholesterol in plasma is, however, not clear. Thirty per cent of this plasma cholesterol occurs as free cholesterol, part of which will be esterified and become cholesterol ester. This continuous process is caused by the action of LCAT. This enzyme, which is formed in the liver, converts fatty acids from the β-position of lecithin into cholesterol according to the reaction.


Lecithin + unesterified cholesterol $\xrightarrow{LCAT}$ cholesterol ester + lysolecithin.

This reaction occurs in α-LP only. The metabolic abnormalities, with elevated plasma triglycerides and pre-β-LP in the rare disease of LCAT defi-

ciency indicate that a normal LCAT reaction is a prerequisite also for the normal pre-β-LP and plasma triglyceride metabolism (14).

Xanthomatosis has frequently been stressed as a characteristic clinical finding in hyperlipoproteinemia. In a population sample xanthomatosis may be expected in less than 1% of the subjects investigated. Tendinous xanthoma, when found isolated, strongly suggests the presence of hereditary hyperlipoproteinemia of type II A. Cutaneous xanthomas, on the other hand, are frequently found in hyperlipoproteinemia type III and then often in combination with tendinous xanthoma as well as with plantar and palmar xanthomatosis. Hereditary hyperlipoproteinemia occurs in subjects even in the absence of xanthomatosis.

The importance of genetic factors in hypercholesterolemic CHD associated with xanthomas has been recognized since the pioneer work of Muller and Harbitz. Data demonstrating genetic influences in a variety of hyperlipoproteinemias are continuously accumulating. Nevertheless, genetic aspects are often overlooked in studies of hyperlipoproteinemia and CHD. With the information available at present it would seem futile to try to evaluate the importance of CHD "risk factors" if the genetic influence is ignored.

The underlying mechanism and the nature of the inherited defect are poorly understood for the more frequently occurring types of hyperlipoproteinemia. Data from the Bethesda group (12) indicate that the defect in type II A is one of reduced catabolic rate of β-LP rather than of increased synthesis. This variant of hyperlipoproteinemia follows an autosomal dominant mode of inheritance (at least in many families) and it could be argued that the mutant gene causes a structural change in the β-LP which makes it less accessible to the catabolic processes. However, data reported by Langer et al. (12) indicate that the difference in catabolism of β-LP between normal subjects and people with hyperlipoproteinemia type II A is not the result of inherent differences in the β-LP molecules. On the other hand, Grant et al. (9) have recently reported a difference at the molecular level between normal β-LP and that of patients with familial hyper-β-lipoproteinemia, type II A, inasmuch as the latter was found to contain more water of hydration than the former.

The results of pedigree analyses indicate that no genetic linkage exists between the genes responsible for Lp(a) lipoprotein and hyperlipoproteinemia type II A (3, 15). Therefore, if the apparent relatedness between Lp(a) lipoprotein and pre-β-1-LP of Dahlén et al. (4) as well as the observation by these authors of an association between pre-β-1 LP and CHD are confirmed, it may be hypothesized that the syndrome of hypercholesterolemia, xanthomatosis and CHD is genetically different from the greater number of cases of CHD. The prospect of revealing genetic "risk factors" early in life holds important implications for the attempts to prevent or delay manifestations of CHD and for genetic counselling.

At present, in clinical practice, hyperlipoproteinemia types III and V appear to be causing problems concerning genesis and management. Type V can be found as a reversible form, secondary to moderate alcoholic abuse, and also in people with diabetes mellitus. Other distinct clinical features of type V have appeared: the disease is characterized by recurrent pancreatitis, peripheral neuropathy, diabetes mellitus, obesity and frequently by hepatic steatosis without concomitant alcoholic abuse. This form appears to be fairly resistant to available dietary management and lipid-lowering drugs.

In any type of hyperlipoproteinemia the prescription of a diet should be the first measure in its management. Type II A, hypercholesterolemia, is improved by a reduction in total fat content in the diet and a change to polyunsaturated fat. In types II B, III, IV or V the goal should be a reduction of body weight by a reduced caloric intake in addition to increased physical activity and it is advisable to substitute polyunsaturated fat for saturated. Experience shows that it is not sufficient to supply the patient with a diet chart only. At the beginning of the dietary treatment there should be an interview in which proper individual advice as to changes in diet is given. Dietary measures alone should be practised for at least four months, during which repeated plasma lipid analyses should be made. If a sufficient lipid-lowering effect is accomplished, dietary treatment should continue. Plasma lipids should be followed and the patient reminded about his specific diet at least twice a year. If no adequate lipid-lowering effect is

achieved with diet alone after 4–6 months, the addition of a lipid-lowering drug might be considered. Even in the combined diet and drug treatment, weight reduction is important for successful plasma lipid reduction, particularly in hyperlipoproteinemia types III and V

It will be apparent from this fragmentary presentation that many questions concerning hyperlipoproteinemia and CHD remain unanswered. Recent developments indicate that it would be wise now to give high priority to research concerning the molecular and genetic aspects of this relationship.

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Acta med. scand. Vol. 193 pp. 373–378, 1973

A NEW HEMODIALYSIS CONSOLE

I. *General Description*

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Abstract. A general description of a new single-patient hemodialysis console is given. The present model, which is designed for centre dialysis, is constructed to ensure a high degree of safety in the dialysis procedure and several consoles can be combined to form an integrated warning system, which should allow a reduction in staff. Serious events in the procedure cause automatic interruption of dialysis. The console can be sterilized by heat and by chemicals. Leakage of blood into the blood pressure gauge is prevented by the use of disposable hermetic blood pressure transducers. Individual functions of the console are manufactured as separate modules, which can easily be replaced, facilitating a speedy repair.

In the late forties hemodialysis became applicable to clinical use and was introduced in the treatment of acute renal failure and intoxications (1, 8). With the development of the arterio-venous shunt in 1960 (14) it became feasible to perform long-term repeated dialysis treatment in patients with irreversible renal failure (6).

Very soon it became evident that this treatment was very exacting in respect of the quality and quantity of the staff, the economy and the equipment, if good results were to be obtained (9, 13, 15).

In view of this a Danish committee on renal hemodialysis was set up in 1965 under The Academy of Technical Sciences, as it was considered that the equipment could be improved.

The purpose of this paper is to give a general description of the first result of the work—the construction of a hemodialysis console. In a following paper some constituent parts will be described in greater detail (4).

Hemodialysis systems can be divided into two main groups with respect to the production of dialysis solution.

1. Plants with central preparation of dialysate (2, 11).

2. Plants with preparation of dialysate in the individual consoles a) as one ready portion before the treatment (12) b) as continuous mixing of concentrate and water during the treatment (16).

The last mentioned system (2 b) was chosen for the following reasons:

1) It is possible to individualize the composition of the dialysing solution according to the needs of the patient.

2) In case of failure of the equipment only one dialysis will be interrupted.

3) With small modifications the equipment is suitable for home dialysis and do-it-yourself dialysis.

4) From a bacteriological point of view it is a great advantage to keep the period from the production of dialysate to its use in the dialyser as short as possible (7).

The present hemodialysis console may be divided into three functional parts, one for the preparation and handling of the dialysis solution (hereafter called the water system), one controlling the extracorporeal blood circulation (hereafter called the blood system) and one including the warning devices in the water and blood systems (hereafter called the warning system).

WATER SYSTEM

The principle of the water system in the console is seen in Fig. 1. Pure water produced either by

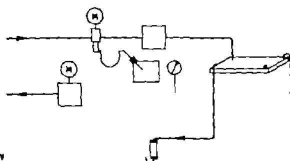


Fig. 1. Simplified diagram of the ater and blood systems.

distillation (5) by reverse osmosis (10) or by deionization, is mixed with the concentrate in a proportionating pump. After thermostating to the correct temperature the dialysate moves on through the dialyser. The pressure level of the dialysate is set by a pressure regulator located between the dialyser and the drain.

Fig. 2 illustrates all components of the system. The pure water passes a filter (*1*) which retains all particles which may cause disruption of the membrane in the dialyser and/or damage the moving parts in the console. The water pressure is reduced to a convenient level in a reduction valve (*2*) preheated to about 35 C and deaerated in the container (*3*). A warning contact with an alarm in case of insufficient water supply is present in the receptacle (*3*). The water is mixed with concentrate (*5*) from the container (*6*) in proportion 30+1. After mixing, the dialysate passes through a safety valve (*7*) which opens in case of an obstruction in the system after the mixing pump (*4*) thus preventing an untoward pressure increase in the water system. Another valve (*8*) prevents negative pressure, established by the pressure regulator (*20*), from reaching the mixing pump since suction on the outlet could cause irregular pumping. After a second heating to 38 C (*9*) and further deaeration the conductivity (*10*) and temperature (*11*) are checked. The

Fig. 2. Complete diagram of the water and blood systems, showing all components. (For explanation, see text.)

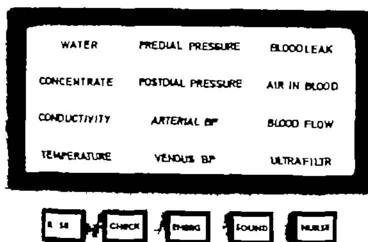


Fig 3 Close-up of the alarm panel

systems of controlling, warning and reading are totally independent. The dialysate pressure before the dialyser similarly has a reading (*13*) and a pressure warning (*14*) which will stop the mixing pump, heating units and pressure regulator if the pressure exceeds the preset limit.

A recirculation pump (*16*) is inserted between the in- and outlet of the dialyser (*15*) and permits the use of a high local flow in the dialyser. A pressure control with reading and an independent warning function with preset limit (*17*) is located on the outlet line. A pipe (*18*) in the outlet line is used for sterilization of the concentrate line (*5*) as described later. A blood leak detector (*19*) is situated on the line just before the pressure regulator (*20*).

BLOOD SYSTEM

The *blood system is shown in detail in Fig. 2.* The blood is recirculated from a shunt or a fistula marked by an *A* (arterial outlet) and a *V* (venous return). Shortly after the blood has left the arterial *outlet, heparin is added by a micro-infusion pump* (*21*) to prevent coagulation of the extracorporeal blood. A blood pump (*22*) is used if the blood flow is insufficient. The blood pressure is measured with disposable hermetic chamber (*23*) which transfers the pressure to the pressure gauge. If the pressure exceeds fixed limits, a warning occurs and the pump (*22*) is arrested.

The use of disposable units is of considerable importance as it prevents leakage of blood into the blood pressure module. Blood contamination of conventional connecting pieces to blood pressure transducers represents a potential source of cross-infection—especially with hepatitis virus.

After the dialyser the blood passes a disposable blood flow meter (*24*) with adjustable warning limits. The bubble trap (*25*) is fitted with a blood level control with alarm in case of a dangerously low blood level. A disposable pressure transducer (*26*) similar to that on the arterial line and equipped with adjustable warning limits, is seen after the bubble trap. In case of air bubbles in the blood returning to the patient a bubble detector (*27*) *clamps off the blood stream* and stops the blood pump.

PATIENT SAFETY AND WARNING SYSTEMS

During the treatment a high degree of safety for the patient must be demanded (5). Considerable *problems arise when great numbers of patients* are treated simultaneously in a centre. In this situation a large part of the staff's time is occupied by assistance in starting and terminating dialyses. This necessitates a proper control and warning system, which enables the staff to have immediate and sufficient knowledge of the performance of all dialysis consoles irrespectiv-

the location and occupation of the individual staff member. The warning system must be constructed in such a way that the necessary information is issued correctly and that a missing function is detected at once if some failure occurs in the warning system. This has been obtained by applying the following principles:

1) All relays in the critical alarm systems are activated during smooth function. The moment a warning device is triggered or fails, the alarm relay is inactivated and an alarm is thus issued.

2) All critical parameters with respect to the safety of the patient are double-controlled.

3) All critical systems consist of independent functions for a) control, b) monitoring and warning, and c) reading. The calculated risk of having simultaneous failures of independent functions is very low. By built in routine check facilities an unnoticed failure in a non-critical function will be detected early.

4) With the exception of blood leak into the dialysate all high priority alarms (see below) are associated with automatic interruption of the dialysis.

5) Each console is equipped with a warning panel giving information on *all* other consoles in use through an interconnecting system.

6) During smooth function all bulbs in the warning panels glow faintly which enables a check on the function of the bulbs.

7) When an alarm is issued, the bulbs flash continuously since all the alarms are built with ...ter function (i.e. even if the warning limit is exceeded only momentarily the alarm remains until reset). The nurse will have to go to the console in question to eliminate the alarm. It cannot be reset from another console.

8) No installed warning system can be shut off or adjusted by members of the staff. Such an operation requires opening of the electronic compartment and can only be carried out by specially trained personnel. This precaution was deemed to be of paramount importance since frequent adjustments carried out by technically untrained staff endanger the integrity of the entire system.

An analysis was made of all possible complications which could occur during dialysis, and this analysis resulted in the selection of a number of parameters to be used in the supervision of the procedure.

Each parameter is shown on a warning panel and is allocated a symbol (e.g. blood flow) and a priority (high = red light, low = blue light) indicating whether the nurse should act immediately or within a few minutes.

Fig. 3 shows the warning panel of the console.

As mentioned, all consoles can be interconnected to form an integrated alarm system. When an alarm is issued *every* warning panel flashes: 1) a parameter (e.g. venous pressure) 2) a priority (in this case high = red light) 3) a station number (e.g. station 7).

A nurse with another patient, possibly in another room, will know that something is wrong at station 7 with an alarm on the venous pressure (and that the blood pump has stopped).

In this way every staff member can move freely from patient to patient and yet be informed of the situation at every station.

With a main reversing contact on each console the warning signals can be confined to the local station to avoid unnecessary loading of the integrated system. The parameter signal will then show its "own" station only while priority and station number are still received from other stations. When the nurse leaves the patient, she reconnects the console to the integrated system by turning the main reversing contact.

If two stations issue an alarm simultaneously the staff cannot directly know from the warning panels which alarm comes from which station. By pressing the button indicating one of the station numbers in question it is possible to suppress all other warning signals than those coming from the station concerned. By selective successive suppression of the warning signals the origins of the alarms can be quickly distinguished.

At any time during a dialysis a check button enables the staff to ensure that the interconnections and the transmitters in the integrated warning system are correctly functioning. By pushing simultaneously the check button and a station number button, the parameter, priority and selected station number lights will flash.

STERILIZATION

In the *blood system* only presterilized and disposable items are used (including disposable blood pressure transducers and disposable blood flow meter).

Sterilization of the *water system* between two



Fig. 4 Front of the console, illustrating the position of the concentrate tank (lower half) and 11 replaceable modules (upper half)

occasions of use is important to prevent bacterial growth in the dialysate, leading to unpleasant pyrogenic reactions, sometimes combined with bacteriemia.

In a centre where equipment is used on several patients the great threat is cross-infection, especially with viral hepatitis

The console offers two methods of sterilization.

Heat sterilization is accomplished by connecting the water and drain lines and uniting the connections to and from the dialyser This closed circuit is heated to 80 C by the heating units while the mixing pump recirculates the hot water A special problem arises in the sterilization of the concentrate line The suction tube which is placed in the concentrate container during dialysis must be clean and sterile both on the outer and inner surfaces. Prior to sterilization the tube is placed in the slanting pipe (*18* in Fig. 2) which is incorporated in the water system.

Chemical sterilization is performed (e.g. with formalin) either by suctioning the concentrated sterilizing agent through the concentrate line and recirculating the dilution as mentioned above, or by suctioning the ready diluted agent up through the water inlet until the whole system has been flushed. The sterilizing agent is left in the machine from one period of use to another

As the sterilizing agent is put into a closed system, a combination of the two methods is possible (and actually used) without any discomfort for the personnel.

MAINTENANCE AND REPAIR

Although the systems are designed for low frequency of maintenance on the console no system can work trouble-free indefinitely The complexity of a system creates obstacles in repairing the faulty parts. Therefore the system is split up into subsystems which are well defined plug-in units built as modules with associated printed circuits. In the hospital a defective console can thus be quickly restored to use merely by replacement of one or more subsystems while the actual repair job is done by the manufacturer in his workshop.

Fig. 4 shows the front of the console and illustrates how the individual functions are mounted as eleven replaceable modules.

All moving parts are constructed and insulated in a manner which permits, practically speaking, noiseless performance.

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Acta med. scand. Vol. 193 pp. 379–385 1973

A NEW HEMODIALYSIS CONSOLE

II. *Descriptions of Components*

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Abstract. A description is given of some constituent parts in a new single-patient hemodialysis console. The parts, which are all developed especially for use in hemodialysis, are: proportionating pump, inductive conductivity meter, heating and deaerating system, blood leak detector, pressure regulator, micro-infusion pump, disposable blood pressure transducer and disposable blood flow meter.

In cooperation with technicians and physicians from the Technical University of Denmark, The Atomic Energy Commissions Establishment, Risø and Rigshospitalet (University Hospital of Copenhagen), the Danish Committee on Renal Hemodialysis has developed a new single-patient hemodialysis console (2). After a period of clinical trial the console (produced by the Danish Sugar Co.) was taken into production and some further improvements were made. The purpose of this paper is to describe some constituent parts in greater detail.

HEATING AND DEAERATING SYSTEM

Considering the safety and comfort of the patient the temperature regulation of dialysate must be accurate and exhibit good performance. An increase in temperature can result in hemolysis which may be life-threatening, e.g. due to hyperkalemia (1). A decrease in temperature is rarely harmful (2). It may however give rise to physical discomfort and may sometimes result in spasms in the return vessel of a shunt (or fistula), thus tending to reduce the blood flow through the dialyser.

On heating of dialysate the solubility of air decreases. This leads to an increased partial air tension in the dialysate, which may be harmful in two ways: 1) Decreased efficiency of the dialyser due to bubble formation on the membrane, a factor which becomes accentuated when dialysate pressure is kept at low levels for purposes of ultrafiltration. 2) The increased tension of air may lead to diffusion across the dialysis membrane and give rise to the clinically well known microbubbles in the blood returning to the patient.

Two-step heating combined with effective deaeration after each heater is used to ensure precise heating combined with effective deaeration. The liberated air is shunted past the dialyser to a position just before the pressure regulator. The heating units are similar in construction and each has an output of 1.5 kW. The liquid is led along a heating rod in a narrow slot. The heaters are regulated on-off via thermistors in the outflow. An integrated operation amplifier is combined with a triac function as amplifier and contact unit. When starting the system up from cold, it is a matter of minutes before the dialysate reaches a temperature of 38 °C (±0.1).

Minor temperature fluctuations are smoothed out by flow of the liquid through two small vessels, which also damp pressure fluctuations arising from the action of the mixing pump. These vessels allow the above mentioned two-step deaeration and, furthermore, they contain gauges for detection of the following parameters: temperature, conductivity and failure of water supply.

Monitoring, read-out of temperature as well as warning in case of failure are three separate and independent systems. The warning system consists of a mercury thermometer with permanent

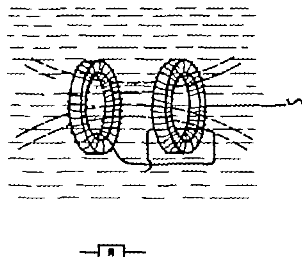


Fig. 1. Principle of the conductivity measurement.

platinum contacts, giving warning at a deviation of ±1 C and interrupting heaters and pumps at a deviation of ±3 C.

The prototype of the heating unit was developed in the Construction Department at Risø (6).

INDUCTIVE CONDUCTIVITY METER

In dialysis it is of paramount importance to maintain a correct concentration of the used salts in the dialysate, as both too high and too low concentrations may lead to serious damage to the patient (10). When using a system with continuous xing of concentrated salt solution with water permanent measurement of the concentration cessary. The usual method of concentration measurement in dialysate is based on the impedance of the solution, using submerged electrodes. However if the liquid is supersaturated with air bubbles may settle on the surface of the electrodes, resulting in an apparently lower and unstable conducti ity. Other methods, such as specific gra ty measurement or refractometry are of doubtful value for this purpose.

Thus a method based on an inducti e measurement seems very attractive as it is primarily sensitive to the amount of surrounding water. Small air bubbles in the water or on surfaces have only an imperceptible influence on the impedance in the total water volume.

The conductivity of dialysate is ery dependent on the temperature and changes by about 1.7% / C in the range of 38 C. Normally a complete temperature compensation is required but, for hemodialysis, compensation only in the vicinity of 38 C seems reasonable, as the temperature of the liquid is well regulated. A limited type of compensation is cheap and, furthermore, provides an extra safety in case the multiple temperature safety warnings should fail, since an extreme deviation of about 5-6°C will also be detected as a deviation in the conductivity.

The principle of the measurement is shown in Fig. 1. The transducer which is submerged in a small receptacle, consists of two toroid coils acting as transmitter and receiver. The coils are magnetically isolated from each other to minimize the leakage flux. The saline acts as the electromagnetic coupling between the coils. A high frequency AC is passed through the transmitter. The saline coupling is outbalanced at the correct salinity by positioning a counter-coupling between the two coils and providing it with an appropriate resistance. Deviations of an order of magnitude of 1 1 000 in the saline concentration can hereby be detected when a suitably high transmitter frequency is used. A phase-sensitive detector discerns a positive or negative deviation.

A 2% deviation from the preset concentration values results in a warning and a 5% deviation sets off the alarm with automatic interruption of the dialysis. The transducer with coils and counter-coupling is covered with a water-resistant, non-toxic resin to protect the components against moisture, chemicals and heat.

The principle of this measurement and of the first prototype was developed by J. Diamond, Electronic Dept., Risø (4).

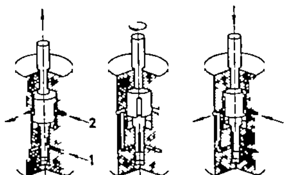


Fig. 2. Principle of the proportioning pump.

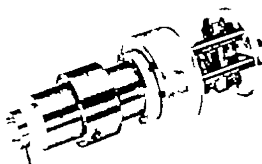


Fig. 3 The assembled mixing pump

MIXING PUMP

The concentration of salts in the dialysate must be well defined and this implies that the propor tionating system must be characterized by reliable and exact mixing of the relative olumes of water and concentrate.

It must be emphasized that a total failure, i.e. interrupted delivery of both water and concentrate, is a quite safe situation for the patient. The danger occurs if proportionating is faulty the possibilities of which may be divided into two main groups:

1 ( ) Failure of the displacement function (e.g. piston failure) of the water or the concentrate compartments. (*b*) Failure of the valve function of the water or concentrate compartments.

2. Failure in the water or concentrate supply system.

Possibility 2 is independent on the type of mix ing pump, and consequently one must a oid this type of failure by other means. This has been done by fitting warning systems on the water line and on the concentrate line, checking that the water level in the first receptacle and the chord of concentrate in the supply line is of an acceptable order of magnitude.

The correct function of the al es and the piston is obtained by interlocking their movements as shown in Fig. . All functions are established by the complex movements of the piston. The alve function is established by rotating the piston until the milled grooves fit the holes in the cylinder wall. The pumping function is established by moving the piston up and down. Fig. 3 shows the pump with the mechanism which con erts a rotating movement into the described complex movement. Apart from leaks as a result of wear incorrect mixing can only occur if the piston fractures.

BLOOD LEAK DETECTOR

The unnoticed rupture of the dialysis membrane leading to a severe loss of blood through the dialysate, is a grave risk in hemodialysis. Although the majority of membrane leaks are de tected by testing the dialyser before use, severe leaks occasionally occur at variable intervals after start of dialysis. Before construction of the blood leak detector it was unknown to us whether imperceptible leaks occur and cause a continuous loss of blood during dialysis. Therefore the blood

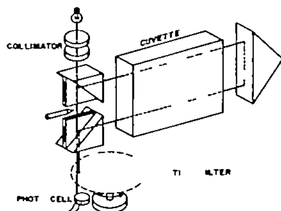


Fig. 4 Principle of the blood leak detector

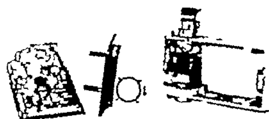


Fig. 5 Cuvette and electronics for blood leak measurements.

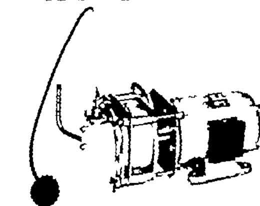


Fig. 7 The assembled pressure regulator (effluent pump).

leak detector was constructed so as to be sensitive to minute concentrations of blood in the dialysate. A photometric method based on the well known maximal light absorption of hemoglobin at 578–565 mμ was used. The principle of the detector is shown in Fig. 4

The filtered light path is split and united again through two double prisms. One path passes through the cuvette containing dialysate and the other passes through a split where the light intensity is adjusted. Through a rotating shutter a photoelectric cell alternately detects the two light paths (measurement and reference path). By adjusting the intensity of the reference light some- higher than that of the measurement path, otocell will deliver alternating voltage de- ding upon the difference between the two

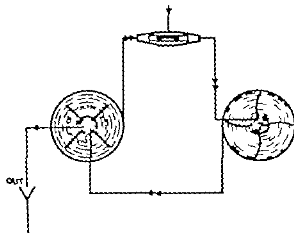


Fig. 6 Principle of the pressure regulator (effluent pump)

light intensities. The imbalance grows if blood appears and the amplitude of the ripple increases. This amplitude is a well defined function of the blood concentration. The signal is amplified, rectified and filtered. A comparator circuit triggers the warning if an upper or lower level is transgressed. When the lower level (zero) occurs, it is a result of failure of components in the detector (e.g. light source, rotating shutter or photo cell). Hereby the correct functioning of the module is ascertained.

At first a warning level of 2.5×10^{-4} blood in dialysate was chosen, but this resulted in the occurrence of too many warnings (caused by small air bubbles and other minute deposits on the windows in the cuvette).

The final level selected was 5×10^{-4} as clinical testing indicated that any leak above this level seems to develop into a large leak, whereas smaller ones (i.e. pinholes) tend to close up within the first hour of dialysis (3). Fig. 5 shows the unit with matching electronics.

PRESSURE REGULATOR

Overhydration of the patient between dialyses necessitates removal of excess fluid during dialysis. The patient better tolerates a gradual loss of water over most of the dialysis period, since an abrupt loss will result in acute hypovolemia. A strong negative pressure and an undulating pressure may result in rupture of the dialysis membrane by fretting. The established negative pressure must thus be held non-fluctuating at the adjusted level

and maximum suction must never exceed the safety limit.

Because of this last requirement the pressure regulator was built as a centrifugal pump. However a simple centrifugal pump will not function satisfactorily because it also receives the admixed air originating from deaeration at the low pressure and from small leaks in the kidney and its connections. Therefore the construction has been based on a two-step centrifugal pump with a high local *recirculation* as shown in Fig. 6. The discharge pipe is inserted in the local recirculation circuit in combination with a bubble separator which removes the admixed air together with the excess dialysate. Thus it is possible to obtain a stable function of the centrifugal pumps, even with admixture of up to 50% air. The pressure regulation is established by placing the inlet between the two pump sections in a twin valve, which gradually can open into one section while simultaneously closing the other. The pressure in the inlet can thereby be adjusted continuously from −400 mmHg to +150 mmHg. The assembled pump is shown in Fig. 7.

MICRO-INFUSION PUMP

In establishing an extracorporeal circuit it is necessary to heparinize the blood. Side-effects such as bleeding (8, 13) and postdialytic hypercoagulability make it necessary to inject the proper amount of heparin within quite narrow limits. High accuracy is needed if a pump provides continuous infusion of small amounts of heparin.

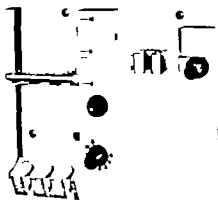


Fig. 8. The front of the micro-infusion pump. The module for blood flow is seen on the right.

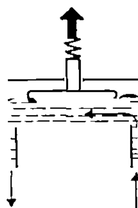


Fig. 9. Principle of the blood pressure transducer.

An infusion pump was developed using disposable syringes to deliver in the range 0.5–5 ml/h. Standard solutions of heparin (5 000 IU/cm³) as well as diluted standard solutions can be used at choice.

The precise movement of the piston is established by a stepping motor which receives impulses from an electric circuit. The motor turns a spindle, which pushes the piston down. Eleven different frequencies are available. The pump is shown in Fig. 8. Through a little window twinkling reflections from a small rotating mirror indicate that the driving shaft is moving and the slow-motion pump is functioning correctly.

DISPOSABLE BLOOD PRESSURE TRANSDUCER

It is a general opinion that a continuous measurement of the blood pressure in the extracorporeal circuit is necessary during treatment. A number of complications which endanger the safety of the patient can hereby be discovered at an early stage if the measurement device is fitted with adjustable warning limits. These complications can roughly be divided into three groups according to the site of complication.

1. Patient. (*a*) Increasing or decreasing blood pressure (hypertension, shock). (*b*) Improvement or worsening of the shunt or fistula conditions (position, venous spasms, clotting, etc.). (*c*) Shunt or fistula disconnection.

2. Extracorporeal [illegible] ance (e.g. clotting of bl[illegible]

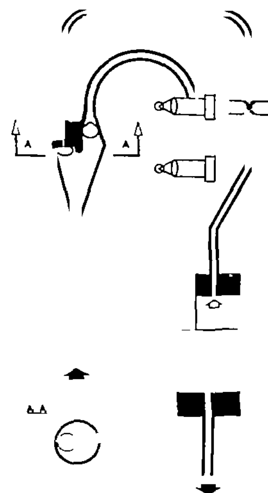


Fig 10 Principle of the blood flow meter

lines) (*b*) Decreased resistance (e.g. disconnection of blood lines).

3 Transducer (*a*) Failing or blocking of the transducer (*b*) Defective mounting.

The usual way in which the blood pressure is measured during hemodialysis is by connection of a Boudon type of manometer to the extracorporeal circuit with a T or Y-ramification on the line. If however a high blood pressure or a slight leak in the leg of the ramification is present, a column of stagnant blood will develop and this might give rise to clot formation. If blood reaches the manometer connections, which are very difficult to clean and sterilize, the manometer may become contaminated and cross infection—especially with hepatitis virus—may become a serious threat to the patients.

A disposable through-flow transducer is thus a good solution. The construction is seen in Fig. 9 The transducer forms a housing with tube connections for the in- and outlet of the blood. The housing is divided in two compartments by a flexible membrane, one in which the blood flows and another in which a flat piece of plastic has contact with the membrane. The pressure on the plastic is transferred to the blood pressure gauge and is easily converted to a suitable electrical signal. The transducer is mounted in the blood line, forming a sterile and disposable set. The transducer is manufactured by the Danish Sugar Co.

DISPOSABLE BLOOD FLOW METER

A reliable blood flow measurement is a valuable aid in hemodialysis. Continuous measurement is especially important when the flow is declining or already low A routine measurement could give an indication of the shunt's state of function" and help in planning the time needed for sufficient hemodialysis.

If moving parts are in contact with the blood, they must be specially surface-treated and have a very fine finish. Fibrin deposits on the surface are a frequent occurrence and give rise to errors of measurement and to clot formation. Several principles, in which movable parts are avoided, ha e therefore been applied in the clinic. The electromagnetic flowmeter (7 11), the Dobbler flowmeter (12) and the hot-film flowmeter (9) are the most commonly used equipments. They have however some drawbacks in common.

1) Their relative precision is low at low liquid velocities 2) The equipments are quite expensive and require precise calibration. 3) The transducers are non-disposable, difficult to sterilize and can give rise to cross-infection (hepatitis virus).

In hemodialysis a widespread method for simple blood flow measurement is to count the seconds it takes for an air bubble injected with a syringe to pass along a known length of blood tubing of known caliber It is very lenient to the blood and gives a check of the flow but is not suited for continuous supervision.

Based on this bubble method an automatic flow meter for continuous measurement of flow was constructed. The sterile air in the bubble trap on the venous return is introduced into the blood some way upstream through a special probe as

shown in Fig. 10. This probe is placed higher than the bubble trap. The pressure in the latter is higher than in the probe since the hydrostatic pressure increase is higher than the pressure drop *due to flow* between the two parts. A tube leading air from the top of the bubble trap to the probe gives rise to the production of uniform bubbles *of suitable* size. The blood flow is measured by the transit time between two photocells.

A low bubble frequency is desirable for two reasons: 1) A high bubble frequency will result in too high blood flow readings (especially at low flows) due to the volume of air added to the blood stream. 2) A high bubble frequency increases the tendency to foaming in the bubble trap. A low bubble frequency is obtained by an adjustable peg on the air tube.

The blood flow meter exhibits a constant relative precision at all rates of flow. Thus the absolute precision is best at low flow rates, at which reliable measurement is most important in clinical use.

The blood flow meter is cheap and is mounted in the venous blood line, forming a sterile and disposable set.

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Acta med. scand. Vol. 193 pp. 387–392, 1973

LARGE SCALE PRODUCTION OF STERILE, DISTILLED WATER FOR HOSPITAL DIALYSIS

Description of Equipment and Evaluation of the Water Quality

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Abstract. An all stainless steel equipment for production and distribution of distilled and sterile water at the rate of 500 l/h to a 9-bed hospital hemodialysis unit is presented. A 2 year trial period has proved that the product water is of high chemical purity, permanently sterile and free from pyrogens, fulfilling the requirements for aqua purissima sterilisata. Excluding depreciation of the installation, the water is produced at a price of D.kr. 9.36 (equivalent to US $1.37) per 1 000 litres.

An important problem in hemodialysis treatment is to obtain sufficient amounts of high quality water for the production of dialysate. High calcium contents in the water may result in hypercalcemia, i.e. cause hard water syndrome (11, 13) and the use of non-physiological calcium concentrations in the dialysate over a lengthy period promotes bone disease and metastatic calcifications (16, 22, 23, 30, 31). High fluorine content induces the risk of fluorosis and fluorine intoxication (12, 27). A fluctuating content of electrolytes is unwanted partly because it complicates the mixing and controlling of dialysate and partly because it involves the risk of exposing the patient to toxic concentrations of ions (5, 15, 18, 19).

Although experiments indicate that bacteria and proteins do not usually penetrate the dialysis membrane (3, 4), pyrogenic reactions have been observed in the clinic (4, 6, 4, 29) and may be explained by the use of polluted dialysate in the presence of pinholes in the membranes. Consequently the dialysate ideally ought to be of infusion quality. This means that the water should be pure, without pyrogens and germs. Ideally the same standards ough to be fulfilled by the added chemicals.

Water purification is usually performed by one of the following methods.

a) *Purification through exchange resins.* This must often be followed by a filtration through a coal and/or asbestos filter to remove pyrogens. The resins frequently become reservoirs for extensive bacterial and mycotic growth, which is extremely difficult to suppress permanently (25). By using warm water (50 C) the growth can be inhibited (7), although other, more heat resistant species, may move in on the resins. The resins become progressively less effective with use and require regeneration at regular intervals. Serious complications have been observed when the chemicals used for regeneration are not properly removed (19).

Electrodialysis (26) exhibits the same disadvantage as exchange resins since it removes neither particles nor molecules which are unloaded or large.

b) *Purification through membranes* (*reverse osmosis*) is a better method, since it purifies water to a considerable extent (2, 17). If not used continually, sterility cannot be maintained (17). Hot water cannot be used in this system since separation becomes less effective with rise in temperature. The membranes become progressively less effective with use and must be replaced at regular intervals. Permanent monitoring of the produced water is necessary for this reason as well as for the prompt registration of membrane rupture. Despite these problems, "reverse osmosis", when properly used, appears to fulfil the practical requirements for production of good quality water for dialysis purposes (17, 28).

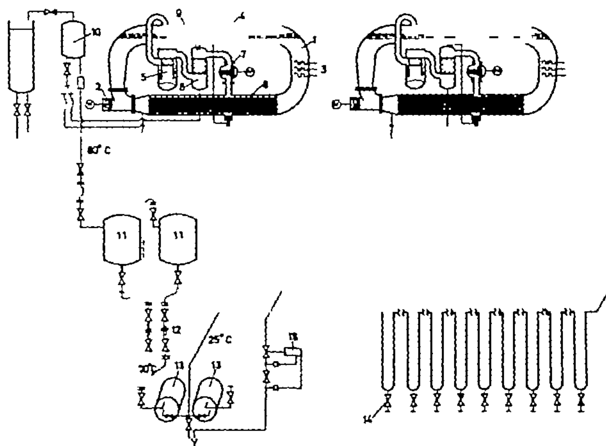


Fig. 1. Schematic illustration of the distillation system and the water distribution system.

c) *Distillation of the water* should theoretically be the ideal method. However distillation must be performed on a large scale before it is economically feasible for this purpose (14).

At the University Hospital of Copenhagen a water supply system built of stainless steel for nine beds based on distillation was installed in 1970. The purpose of this paper is to report on its construction and the results of its use.

GENERAL REQUIREMENTS

In the construction of the plant a number of requirements were made.

The *water production system* must have a production of water in abundant amounts also at periods with maximum dialysate requirement, be constructed for prompt repair or replacement in case of breakdown and have a low running consumption of power and maintenance.

The *water distribution system* must be designed to work under sterile, preferably autosterile, conditions and be readily sterilizable in case contamination should nevertheless occur.

The *product water* must be free from solutes, germs (including virus) and pyrogens.

DESCRIPTION OF THE PLANT

Fig. 1 shows a diagram of the plant. The distillation equipment, the reservoir tank (*11*), the pipeline (*12*) and the cooler (*13*) are duplicated. Each of these elements can easily be substituted by its duplicate or used simultaneously.

The distillation apparatus and the reservoir tanks are located in a pent-house at the top of the hospital. The hot distilled water is continuously brought down to the dialysis unit where it is led via the cooler through a single delivery line directly to the bed stations. At the end of the delivery line a flow regulation valve (*15*) monitors a constant drain of water (1–2 l/min) indepen-

dently of the rate at which water is used at the nine bed stations (*14*). Hereby a continuous flow through the whole system is ensured. This means that the water arriving at the heat exchanger is always hot and that stagnation of water in the pipeline as well as bacterial contamination from the drain is inhibited.

The stainless steel material used in the system is type SIS 142343 which contains Fe (64%) Cr (17.4%), Mn (1.7%) Ni (13.5%) Mo (2.7%) C (<0.05%)

Distillation equipment

The distillation equipment was designed and constructed by R. Andersen, Kemiterm, Gentofte, Denmark (1). Warm tap water heated in a heat exchanger to about 100 C as described below is continuously led to the 0-shaped brine section (*1*). A 10% excess of tap water is used to counteract concentration and sedimentation of solutes in the brine. The brine is vigorously circulated by a pump (*2*) and heated (*3*) to a temperature of 103 C. Saturated water vapour is present at the top of the brine section (*4*). This vapour is led via a cyclone (*5*) where all particles, including condensed drops, are removed, and through a deaerator (*6*) in which dissolved air is removed from the distillate (*9*) in countercurrent with the vapour. This is then compressed in the compressor (*7*) and condensed in the heat exchanger (*8*), which is located inside the brine section. The distilled water is led through another heat exchanger (not shown in the diagram) which brings the temperature of the distillate down to about 80 C and simultaneously heats the arriving warm tap water to about 100 C before entering the brine section as indicated earlier. One apparatus is producing at a time while the other is in stand-by position with a hot brine section. The mean duration at 103 C of the water in the brine section before its conversion to distilled water is more than one hour.

The reservoirs (*11*) consist of two heat-insulated tanks, each containing 1 100 litres. They are ordinarily sealed but can be used as open tanks for batch mixing of dialysate in case of an emergency. One or both receive continuously the hot distillate at a rate of 500 l/h via a small level container (*10*). The surplus of hot distillate is led back to the brine section as water production is normally larger than the consumption for production of dialysate. Thereby the tendency to incrustations in the brine section and in the heat exchanger is further reduced.

The piping systems (*12*) are likewise insulated and direct the hot water down to the dialysis unit.

The coolers (*13*) (likewise only one used at a time) reduce the water temperature to about 25 C. The cooling systems (not shown in the diagram) are located in the pent-house and circulate cold water to the coolers. In an emergency cold tap water can be connected to the coolers if the (doubled) cooling system should fail.

The water distribution line (*14*) to the dialysis stations is the only non-duplicated part of the system. The water is led out to the dialysis stations through a single pipe (as more than one piping system to the stations could lead to fatal mistakes if the system not in use was filled with sterilizing fluid (*10*)). This pipe is constructed as a continuous tube. The valve at each bed station is short-stemmed in order to reduce the volume of potentially stagnating water as much as possible. The valves are of the membrane type without any corners or crevices. After the last bed the pipe ends in a pressure regulation valve (*15*) which maintains a continuous low flow of water through the pipe. This means that the entire system is permanently flushed with pure water and that a minimum flow rate is maintained also when dialysis is not performed.

The dialysate is produced at each station in single-bed hemodialysis consoles containing proportionating systems. This type of console, which is sterilizable by heat and by chemicals, has been described in earlier papers (8, 9). The consoles are connected by sterile lines to the water distribution system.

STERILIZATION

The system after the distillation plants can be sterilized in two ways, by chemicals and by heat.

Chemical sterilization can be performed by introducing the chemical (e.g. formaline 2% w/v) into the reservoir tanks (11) and letting it flow down through the system. This method has not yet been used because the attainment of permanent sterility of the system has not been a problem (see later).

Heat sterilization is only relevant in the heat exchangers and the water distribution system to the dialysis stations. This is done by replacing the

Table I. *Examination of the product water*

	Distillation plant	Bed station
Conductivity μ^{-6} ohm^{-1} cm^{-1}	0.[illegible]5 (range 0.25–0.[illegible]6)	0.28 (range 0.25–0.30)
Evaporation residue of 2 000 ml	< 0.1 mg	< 0.1 mg
Activation analysis Iron	< 0.005 parts mill.	< 0.006 parts/mill.
Flame photometry Potassium, sodium, calcium, iron	Not demonstrable	Not demonstrable
Ultraviolet absorption spectrophotometry (219 mμ–800 mμ)	No trace of organic components	No trace of organic components
Aqua purissima test for metals	Not demonstrable	Not demonstrable

circulation of cooling fluid in the coolers with hot tap water (95 C). Within 15 min the entire pipeline system is heated and thus sterilization can be maintained for any desired length of time.

During the two years' experience it has not been necessary to clean the components (apart from the initial cleaning prior to their use).

THE CHEMICAL PURITY OF THE WATER

Examinations have been carried out on the water drawn at two points: 1) the outlet from the distillation plant and 2) the last bed station.

The results are summarized in Table I. It will be seen that the analyses show barely detectable contents of solutes.

The water from the two points has also been tested by the Pharmaceutical Laboratory of the National Danish Health Service. The permissible levels of metals in "aqua purissima" expressed as parts mill. (20) are: copper < 0.003 chromium < 0.002, ferrum < 0.02, manganese < 0.04 nickel < 0.04 stannum < 0.04 zinc < 0.5. In all instances the levels in the water were below the mentioned concentrations.

Table II. *Estimation of depreciation and running costs*

Specification	Annual sum (D.kr.)	Per 1 000 l (D.kr.)
Depreciation	100 000	22.73 (70.9)
Maintenance	3 050	0.68 (2.1)
Labour	10 500	2.39 (7.4)
Power consumption	[illegible]4 600	5.60 (17.5)
Water consumption	3 040	0.69 (2.1 %)
Total cost	141 190	32.09 (100.0)

THE MICROBIAL PURITY OF THE WATER

From the beginning of its use the system has been regularly tested for microbial contamination. During the first six months samples were drawn from each bed station in sterile 500 ml bottles. Because of almost constant sterility the sampling frequency was reduced thereafter. The 500 ml samples were passed through filters (Millipore® Type HA pore size 0.45 μ) which in turn were placed, contaminated side up, on a substrate (blood-yeast agar). After incubation for [illegible]4 hours at 34 C visible colonies were counted through a magnifying glass. In the case of growth identification of the germs was performed.

In the two years of use 178 tests were made. Six of these showed growth (with less than 50 colonies/500 ml water). As two of these were growth of S. albus (the rest were Pseudomonas species) and the positive tests were not in succession, we believe that at least some of the samples were contaminated either during collection or during the filtration procedure.

TESTING FOR PYROGENS

Three tests for the presence of pyrogens were carried out at the State Serum Institute and were

negative. We have not observed any classical pyrogenic episodes in patients during two years use, nor have we had any trace of sediments or growth in the hemodialysis equipment and tubing.

ECONOMIC CONSIDERATIONS

Depreciation of the installed equipment is estimated at 15 years, although similar types of machinery have performed well for such a period without major repairs. The capital invested being about D.kr 1.2 mill., an annual depreciation of D.kr 100 000 is reasonable.

Maintenance covering materials (oil, acid, spare parts, etc.) and tools has been found to be about D.kr. 3 050 annually.

Labour during the first year (which included the period of starting up the machinery) amounted to about one quarter of a mechanic (D.kr 15 000). In the second year it had decreased below one tenth of his time (D.kr 6 000). An average of the two years amounts to D.kr 10 500 annually.

Power consumption is about 35 kW (22 kW in the producing machinery, 8 kW in the stand-by duplicate and about 5 kW in the cooling system).

Water consumption amounts to about 10% more than the produced amount of pure water as described earlier. The minimal annual production of water is about 4.4 mill. litres.

As shown in Table II the depreciation comes to more than 70% of the annual costs. The running costs per unit of produced water decrease considerably with increasing production, e.g. if the production is doubled (both plants running) the running costs would increase by about 67% (or barely 20% of the total costs).

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Acta med. scand. Vol. 193 pp. 393–397 1973

SCREENING FOR HYPERTENSION IN AN EPIDEMIOLOGICAL STUDY

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Abstract. Screening for hypertension has been done in an epidemiological study of a large group of 5 200 Copenhagen men aged 40–59. Men having BP readings above the limits systolic ≥165 mmHg and diastolic 105 mmHg, and ≥diastolic 110 mmHg alone, were subjected to further study. 196 men were untreated and had BPs above the arbitrary limit set up, 150 of whom were included in the present study. One-third of the patients had manifestations of high BP in heart or eyes. Blood values of cholesterol, uric acid, quantitative lipoprotein electrophoresis, Hb and haematocrit were compared with normotensive controls. No differences between patients and controls were found. All patients had their BP measured twice by one observer, the value being lower in the second than in the first measurement, but in only two patients was the second BP below 140–90 mmHg. Few of the patients had earlier been to see their doctor because of symptoms. The results of the study indicate that hypertension is a common and symptomless abnormality or disease and, as shown in this and other studies, involves an increased risk of cardiovascular complications.

In most cardiovascular epidemiological studies a rather high proportion of individuals are classified as hypertensives (5, 8, 9, 15). Prospective data from such studies have indicated that a casually measured high BP is highly correlated to hypertensive and arteriosclerotic cardiovascular disease or death, the risk being increased linearly with both systolic and diastolic BP (7, 8, 9). Furthermore, results from controlled trials have shown that men with only moderate increase in BP benefit from antihypertensive treatment (17). These results from epidemiological studies and controlled trials raise the question of screening and treatment in population mass-studies.

In this paper some results from the screening of BP values in a population of middle-aged men and a subsequent clinical examination of a sample of hypertensives will be presented.

MATERIAL AND METHODS

The patients examined have been selected from a population of 5 200 Copenhagen men aged 40–59, all employed in large companies. All were first examined at their place of work when participating in a prospectively planned epidemiological study. Methods and preliminary results of this study have been published elsewhere (3, 4). Response in this epidemiological study was 85.5%.

Selection of hypertensive men for further study was done according to the following criteria: BP value measured on the right arm at or above a systolic level of 165 mmHg and diastolic BP above 110 mmHg irrespective of systolic BP value. All men should be untreated at the time of the BP measurement. 196 men fulfilled these criteria.

Four additional men having BP above the limits set up were also accepted for study even though they were being given some antihypertensive treatment. Eight men had earlier received treatment.

Number of patients included. One hundred and fifty men were included in the study according to the above mentioned criteria. The 50 other men were not studied. Reasons for non-participation: Eight men wanted rather to see their doctor than to participate in this study; 42 were not included because of organizational problems at the beginning of the study and later because of limited capacity in the Out-patient Clinic where the study took place.

Measurement of BP was done by the author both in the whole population group and in the men selected for the study. The following method was used. A 12 cm wide and 26 cm long cuff was applied to the subject's right arm with the lower end 3 cm above the antecubital space. Pressure was applied with quick inflation of the cuff to 200 mmHg pressure or at least 30 mmHg above systolic BP. Deflation of the cuff was done with 3 mmHg/pulse beat and diastolic pressure was recorded on disappearance of the sound. The same mercury Sphygmomanometer was used in all measurements. In all patients selected for study BP was measured in both arms and twice in the right arm at the same setting. The last measured value was used in recordings. The first measurement took place at the subject's place of work. All subjects had at least 5–10 min rest, and measurement of BP was done

Table I. *BP at the first and second reading*

Systolic	No. of pats. 1st reading	No. of pats. 2nd reading	Diastolic	No. of pats. 1st reading	No. of pats. 2nd reading
140		7	90		6
141 150		16	91 100		44
151–160	8	17	101 110		38
161–170	18	29	105–110	59	
171–180	32	21	111 120	64	39
181 190	35	23	121 130	21	16
191 200	20	17	131 140	5	4
201 210	18	10	141–150	1	3
211 220	7	8			
221	8	2			
Mean	189.2	178 5		116.8	110.4
S.D.	18.7	22.5		10.8	16 0

Not included men 50: mean systolic BP 176.5, S.D 20.3, diastolic BP 113.1 S D 7.9.

with the subject sitting in chair with the arm placed on a desk.

Re-examination of patients selected took place in the Out-patient Clinic of the hospital 14 days to 4 months after the first examination. All examinations were made in the hospital between 9–12 a.m., the patients having had their usual breakfast. The following parameters were recorded.

1) Venous blood values of Hb, haematocrit, creatinine, sodium, potassium, cholesterol, uric acid. In 86 subjects a qualitative lipoprotein electrophoresis was analysed. 2) ECG recording using multichannel recorder The following leads were used. I, II, III, V_1–V_6. 3) Examination of the eyeground by an ophthalmologist (Dr E. Godtfredsen). 4) X-ray examination of the chest.

Analyses of blood tests was done following the usual routine at the hospital' laboratory ECGs ere read by the author according to the Minnesota Code (9).

Four to ten days after the examinations had been performed the patients were seen in the Out-patient Clinic by the author. A standardized anamnesis was obtained by using questionnaire supplementing a questionnaire used in the epidemiological study A clinical examination was

Table II. *Subjective symptoms in 150 hypertensive men included in the study and frequency of subjective symptoms in the total population examined in the epidemiological study*

	N	of total	of total population
Headache	46	30.6	25 0
Dizziness	29	19 3	—
Palpitations	34	22.6	—
Discomfort in chest at rest	19	12.6	18 0
Angina pectoris	15	10 0	3.1

made, and a repeated measurement of BP using the same procedure as in the first measurement. If treatment as considered necessary this was started using a diuretic or in few cases a β-blocking agent. Whether requiring treatment or not, all patients were advised to see their doctor for control. In a non-selected sample thorough search for a renal or suprarenal cause of the hypertension as made. Results and problems concerning this part of the examination will be published later.

RESULTS

The mean age of the patients was 50.4 years, S.D ±5.4.

BP values at the first and second recording appear from Table I.

BP in the second recording is statistically lower than the value of the first recording, the mean difference being systolic 10 7 mmHg and diastolic 6.4 mmHg ($p < 0.01$ for both).

Two patients had normal BP at the second measurement (below 140 mmHg systolic and 90 mmHg diastolic). Five were borderline cases (systolic 141–159 mmHg and diastolic 91–99) Hypertensive BP readings were recorded for 143 men (above or equal to 160/100). The mean BP of the 50 men not included is shown in Table I. The value is a little lower than that of the men included. The difference is statistically significant (systolic $p < 0.01$ diastolic $p < 0.02$).

Subjective symptoms found which might be related to hypertension appear from Table II.

Only a minority of the patients had any subjective symptoms at all, and none of them had been to see their doctor because of symptoms. The occurrence of headache does not differ significantly from that of the total population for which the prevalence was 25%. Angina pectoris diagnosed from a standardized questionnaire and clinical judgement was observed in 10% which is more frequent than expected on the basis of the total population examined for which the prevalence was 3% (χ^2-test, $p < 0.01$).

Objective symptoms generally accepted as being related to hypertension appear from Table III together with the classification of patients according to the WHO criteria.

It will be noticed that one-third of the patients could be classified as stage II according to the WHO criteria. Four men who had severe hypertensive changes in organs were all feeling well subjectively One of them had earlier received treatment.

Table III. *Objective symptoms related to hypertension in the 150 hypertensive men studied*

	N	% of total
Eyeground changes	26	17.3
FH I	15	10
FH II	7	4.6
FH III	4	1.6
FH IV	0	
ECG findings, Minnesota Code (II L, IV 1–3, V: 1–3)	30	20
Left ventricular hypertrophy (X-ray)	31	20 6
WHO stage		
I	95	63.3
II	55	37.3
III	4	2.0

The laboratory studies of the blood samples are tabulated in Table IV and compared to those of 101 normotensive controls drawn from a random sample of the whole population. As will be seen, no differences between controls and patients exist for Hb, haematocrit, cholesterol or uric acid. (Control values for uric acid were provided by Dr J Lyngbye, who had examined normal Copenhagen men aged 40–59.) Three hypertensives had abnormal serum creatinine values. Qualitative lipoprotein studies in a group of 84 hypertensives showed abnormalities in 18 patients. This does not differ from the 19 such abnormalities found in 96 controls.

Diastolic BP values measured in the Out patient Clinic ha e been compared in men with and without retinal changes, and in men with and without changes in ECG.

The 26 patients with retinal changes had significantly higher values than the patients without, 116.9 mmHg, S.D 14 1 against 108.9 S D 12.1 ($p < 0.01$). Seventeen of these 26 patients with retinal changes had at the second BP measurement diastolic alues above or equal to 115 mmHg. Forty-five of 1 4 patients with no retinal changes had BP alues above or equal to 115 mmHg at the second measurement.

Patients with ECG changes had higher diastolic BP at the second measurement than those without (mean 115.7 S.D. 15 62 against 108 mmHg, S.D 16.23). The difference, however is not significant ($p > 0.05$).

Anamnestic data

A history of hospitalization for coronary occlusion was obtained in 7 men, none of whom had received antihypertensive treatment or came to see their doctor regularly The frequency of earlier coronary occlusion (4 7 %) is higher than expected (χ^2-test $p < 0.001$ the frequency found in the total population was 1.5 %).

A history of renal stone attack was found in 18 of the 150 patients, which is not significantly different from the frequency of 10 % found in the total population.

Ninety-four patients were given treatment after the examination in the hospital. Some have received treatment after a later reexamination and the rest were recommended regular control of BP at least annually

DISCUSSION

Comparisons of prevalence of high BP to that found in other studies will be commented on further in a subsequent communication (3). The frequencies of patients having manifestations of high BP in the ECG or enlargement of the heart at X-ray examination are quite similar to those found by Tibblin in the Gothenburg study of men aged 50 (15) and by Hart in a study of a Welsh community population (5). In the Tecumseh study

Table IV *Results of measured blood values in the 150 hypertensive men studied and 101 normotensive male controls of the same age*

	Patients			Controls		
	N	Mean	S D.	*N*	Mean	S.D.
Hb (mg/100 ml)	143	15.27	1 12	101	15 13	1 04
Haematocrit	139	47 13	3.36		46.79	3.00
Cholesterol (mg/100 ml)	142	268 3	11.06		271 8	48.39
Uric acid (mg/100 ml)	140	6.19	1 26		6 10	1.10
Creatinine (mg/100 ml)	137	1 13	0.42		—	—
Lipoprotein electrophoresis abnormal	86	18 abnormal			96	19 abnormal
Creatinine >1.3 mg/100 ml	3					

(14) roughly one-third of men above 40 years and with hypertension had one of the ECG changes registered in the present study in only one-fifth of the subjects. Some selection bias might have occurred in the present study as only 150 of the 200 hypertensive subjects detected were included. This, however is rather unlikely since the BP of men not included differed only slightly from that of patients.

The number of patients having retinal changes was low. Tibblin (15) found that 50% of patients having a diastolic BP ≥ 115 mmHg had retinal changes. In this study only 8% of those with a diastolic BP ≥ 115 mmHg had retinal changes. Slight differences in diagnostic criteria may account for this difference, as may also true differences between the populations.

Among the 62 patients with a diastolic BP ≥ 115 mmHg, 39 had one or more of the following manifestations of BP: ECG findings, retinal changes or left ventricular hypertrophy.

The number of patients observed having angina pectoris or a history of myocardial infarction was greater than expected. This is in agreement with the findings from epidemiological studies, in which a close relation between coronary heart disease and hypertension has been found (8, 13).

Subjective symptoms in this study were few and so vague that only few of the patients had been to see their doctor because of symptoms. This indicate that the only effective way of finding hypertensive patients is by frequent measurement of BP in daily practice or by screening in a population.

The most interesting finding with regard to the results of blood tests is the perfect normality of the values and the lack of positive relationship to BP. In some other studies a positive relationship to cholesterol values in serum has been found (11). Others, however have found no correlation between serum cholesterol and BP in man (10 15 16). The latter findings are supported by this study.

The qualitative estimation of lipoprotein electrophoresis is a very rough screening procedure, and the lack of correlation only indicates that no gross differences exist between groups. Breckenridge found a positive relationship between elevation of uric acid in blood and hypertension (2). This finding cannot be reproduced in this study which is consistent with findings by Tibblin, who reported no elevation of uric acid in male subjects with high BP values (15).

No strict rules as to whom to treat or not were set up in advance. Some rules, however have been followed. All patients having organ manifestations of high BP were treated, and all patients having a diastolic pressure in the second recording equal to or above 110 mmHg were likewise given treatment. For the remainder of the cases an individual judgement was made, taking into regard age, BP and the presence of other coronary risk factors.

One might claim that the few abnormalities found in the laboratory tests and in the other objective measurements in this investigation were due to the circumstances under which the measurement of BP took place, that the patients selected were not "real hypertensives". This objection finds some support from the fact that the second measured BP in the Out-patient Clinic was lower than the first. But only two of the patients selected in this rough way had completely normal BP in one of the two measurements. One may in fact question the current concept of always paying most attention to the last and lowest measured of a series of daily BPs. Perhaps it may be more relevant to measure BP in people during common daily activities, during or after exercise, than when lying in a hospital bed under so-called basal conditions. A casually measured high BP is an important risk factor in coronary heart disease (7), and a fairly good correlation of casual BP and BP throughout the day has been found (13).

Till now only few large controlled trials with the purpose of evaluating the benefit of antihypertensive treatment have been performed (7 18). In these studies, however a most convincing lower frequency of cardiovascular complications was found in the treated group versus controls, and very few unpleasant side-effects of treatment were recorded. The problems concerning detection, diagnosis and management of hypertensive populations have been discussed and guidelines proposed by a hypertension study group in the USA (6) where the prevalence of hypertension is higher than in most European countries (9).

In conclusion it may be stated that hypertension is a common and symptomless "abnormality" or disease involving an increased risk of cardiovascular complications. The frequency with which this disease occurs in different populations, and

the fact that the treatment is easy beneficial and relatively free from side-effects, make screening for hypertension in European populations an obvious public health measure.

ACKNOWLEDGEMENT

This paper was supported by grants from the Association against Heart Diseases and King Christian X Foundation.

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Acta med. scand. Vol. 193 pp. 399–406 1973

STUDIES ON ARTERIAL AND RENAL VENOUS PLASMA RENIN ACTIVITY IN HYPERTENSIVE PATIENTS

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Abstract. The renin secretion rate of each kidney has been estimated in 26 hypertensive patients during supine rest and during stimulation by means of head-up tilt and slow hemorrhage. The patients were off antihypertensive treatment only for the day of the investigation. Eighteen patients had renovascular lesions and eight unilateral renal disease of other etiology. Ten renovascular patients had undergone operations, five with successful and five with unsuccessful result as judged from the postoperative BP. The follow-up period postoperatively was at least one year. It was found that the plasma renin activity was essentially within normal limits in all patients, but the renovascular patients who were subsequently successfully operated on had higher levels than the other patients. Increased renin secretion could be demonstrated in hardly any kidney during supine rest. During stimulation the involved kidney in the successfully operated group responded with increased renin secretion in all patients, while the contralateral kidney was unresponsive and some patients even demonstrated a negative veno-arterial difference in the renin activity of that kidney. In the unsuccessfully operated group no responsive kidneys during stimulation were found. In the rest of the material eight patients with renovascular lesions and eight with unilateral renal disease from other causes, generally low and unresponsive secretion rates, were *observed*. It is concluded that in renovascular hypertensive disease the estimated renin secretion rate during supine rest was not correlated to the result of surgery while the estimated renin secretion during stimulation appeared to have predictive value as regards the result of surgery. In unilateral renal disease from other causes in hypertensive patients the renin secretion was seldom increased in either the supine resting or stimulated state. No results of surgery were available in these groups.

Increased renin secretion of the ischemic kidney is associated with experimental renovascular hypertension (12). In the current literature it is much discussed whether an increase in the renin secretion of an involved kidney in human hypertension could be used for the preoperative diagnosis and identification of conditions accessible to surgical correction.

The common practice has emerged to express the relative renin secretion rate (RSR) of each kidney as the ratio between the renal venous renin activity of the involved and non-involved kidneys, disregarding the fact that the RSR is the product of the veno-arterial difference of renin and the plasma flow of the individual kidney. A ratio greater than 1.5–2.0 is usually considered diagnostic of unilateral, increased renin secretion and thereby indicates the side of the lesion. Very favorable results have been reported by several authors, but recently it has been found that the failure rate of this ratio to indicate the side of the lesion may amount to anything between 20 and 65% (4, 15). These findings indicate the fallacy of this parameter.

It has also been pointed out that, unless the renin secretion is stimulated, it may be difficult to establish with certainty a net renin secretion (10). During basal conditions the veno-arterial difference in renin activity is of the order of 20% of the arterial activity and the error of the renin assay is seldom less than 15–20%. The renin secretion may therefore be masked for methodological reasons. Applying a stimulus for the renin secretion, such as BP reduction and head-up tilt, a few authors have observed that the renin secretion of the involved kidney in renovascular hypertension may be unmasked (10, 11).

We present here some experience in a series of hypertensive patients in which the RSR has been estimated along with a determination of the ratio of renal venous renin during supine rest and in a stimulated state. The results of these

Table I. *Review of the total material*

AS = arteriosclerosis, RAS = renal artery stenosis, FMD = fibromuscular dysplasia, IRO = intrarenal occlusion of one or more branches, S = saluretics, SB = sympathetic blocking agents, BB = β-blocking agent (propranolol), H = hydralazine, M = methyldopa, A = spirolactone

Group	Pat. no.	Sex	Age (y.)	Diagnosis	Known duration of hypertension (y.)	Current treatment	Operation	Follow-up (y.)	BP postoperatively
1 *Renovascular disease*									
a) Operated (successful)	2	♀	25	RAS bilat. FMD	6	SB + H	Right reconstruction	2.0	Normotensive
	8	♀	50	RAS right total occl. AS	<1	SB + H	Right nephrectomy	2.0	Normotensive
	9	♂	49	RAS bilat. AS	<1	S	Left reconstruction	1.5	Normotensive[b]
	101	♂	45	RAS left AS	1	M + H + S	Left reconstruction	1.0	Normotensive
	108	♀	48	RAS bilat. AS	1.5	M + A	Left thrombendarterectomy + reconstruction	1.0	Normotensive
b) Operated (unsuccessful)	3	♀	42	RAS bilat. FMD	1.5	SB + H + BB	Bilat. reconstruction	1.5	Hypertensive
	6	♀	50	RAS bilat. FMD	4	SB + BB + S	Bilat. reconstruction	1.5	Hypertensive
	12	♀	42	RAS right FMD	4	None	Right reconstruction	1.5	Hypertensive
	103	♂	58	RAS left AS	8	SB + M	Left thrombendarterectomy	1.0	Hypertensive
	104	♀	29	RAS left FMD	7	SB + H + BB	Left reconstruction	1.0	Hypertensive
c) Non-operated	4	♀	50	RAS bilat. FMD	14	SB + H			
	14	♂	48	RAS right AS	14	SB + H			
	16	♀	51	RAS left ?	4	SB + H			
	18	♀	56	RAS right ?	<1	M + S			
	21	♂	56	IRO right ?	2	H			
	22	♂	48	RAS right ?	<1	SB + H + S			
	23	♂	57	RAS right ?	1	None			
	105	♀	50	IRO right ?	<1	SB			
2 *Unilateral renal disease*									
a) Contracted kidney	10	♂	50	Right (pyelonephritis)	3	M + H			
	13	♀	33	Right (congenital?)	9	H + BB			
	17	♀	29	Right (congenital?)	1	SB + H			
	27	♂	60	Right (pyelonephritis)	<1	None			
	106	♂	42	Right (st. post hydronephrosis)	<1	SB + H			
	107	♂	57	Right (st. post ureterolithiasis)	4	BB			
	110	♂	42	Left (st. post. hydronephrosis)	<1	None			
b) Hydronephrosis	24	♀	36	Right	<1	SB + M + H			

Operated on 4 y. previously on the left side with almost no effect on the BP. No renin data available from this admission.
Considerably improved for 6 mo., then BP increased again. Operated upon the right side at another hospital, thereafter normotensive. No renin data available from this admission.

Table II. *Data on patients operated on for renovascular lesions*

Group 1 became normotensive after operation, group 1 b remained hypertensive
R = right, L = left, A = arterial, RRV = right renal vein, LRV = left renal vein

Pat. no.	Procedure	BP (mmHg)	HR (beats/min)	Estimated RPF (ml/min) R	Estimated RPF (ml/min) L	PRA (ng angiotensin/100 ml) A	PRA RRV	PRA LRV	$(V-A)_{PRA}$ diff. (ng angiotensin/100 ml) R	$(V-A)_{PRA}$ diff. L	RSR (ng angiotensin/min) R	RSR L	Renal vein PRA ratio
Group I													
2	Basal	169/117	94	250	250	187	299	181	112	−6	280	−15	1.65
	Tilt	118/69	112			277	968	307	691	30	1 728	75	3.15
8	Basal	210/105	88	25	225	272	775	179	503	−93	126	−209	4.33
	Tilt	138/94	90			476	9 967	347	9 491	−129	2 373	−290	28.72
9	Basal	209/120	80	150	100	405	1 210	1 524	805	1 119	1 208	1 119	1.26
	Tilt	143/90	90			1 809	2 165	9 156	356	7 347	534	7 347	4.23
101	Basal	174/94	82	300	200	506	173	610	−333	104	−999	208	3 53
	Hemorrhage	140/75	—			2 061	815	2 230	−1 246	169	−3 738	338	2.73
108	Basal	198/86	51	300	200	243	90	232	−153	−11	−459	−27	2.58
	Hemorrhage	148/86	82			281	90	581	−191	300	−573	600	6 46
Group 1b													
3	Basal	220/110	70	200	200	115	130	171	15	56	30	112	1.32
	Tilt	160/105	90			114	208	229	94	115	188	230	1 10
6	Basal	180/130	46	140	210	381	778	622	397	241	556	506	1.25
	Tilt	120/70	82			396	594	553	198	157	277	330	1 07
12	Basal	160/110	90	160	240	77	67	72	−10	−5	−16	−12	0.93
	Tilt	140/105	100			73	186	103	113	30	181	72	1.81
103	Basal	209/112	71	125	125	42	39	53	−3	11	−4	14	1 36
	Hemorrhage	210/116	80			62	58	42	−4	−20	−5	−25	0 72
104	Basal	194/109	66	300	200	31	17	61	−14	30	−42	60	3.59
	Hemorrhage	173/101	63			23	4	72	−19	49	−57	98	18 00

determinations have been compared with the results of surgery when this was performed.

MATERIAL

Twenty-six patients have been studied. They were all admitted to the hospital for significant hypertension in conjunction with known or suspected renal disease. The diagnostic work-up included i. v. urography renal angiography isotope renography (¹³¹I-hippuran) and total renal clearance in all cases.

A review of the total material is given in Table I. Eighteen patients had renal vascular disease and eight had unilateral parenchymal disease of various etiology. Age, sex and type of lesion in each patient are given in the Table. T the successfully operated group were assigned patients cured by surgery i.e. no further antihypertensive treatment was necessary postoperatively and the BP was reduced to below 160/100 mmHg. The unsuccessfully operated group consisted of the other operated patients in whom the antihypertensive treatment had to be continued postoperatively.

According to the result of subsequent surgery the material of renovascular lesions was split into those successfully and those unsuccessfully operated upon. Each of these two groups (group 1 and 1 *b*) contained 5 cases, i.e. 10 operated cases in all. The remaining 8 cases with renovascular lesions have not been operated upon (group 1). No one of the 8 cases of unilateral renal disease (group 2) was operated upon.

Current antihypertensive treatment as shown in Table I was withheld only on the day of investigation. No sedatives were given. The patients were given regular hospital diet.

The patients were investigated in the morning after an overnight fast. Percutaneous catheters were introduced into brachial artery and an antecubital vein of the other arm. Priming and sustaining infusions of para-aminohippurate (PAH) were then given in the venous catheter aiming at plasma concentration of 2 mg/100 ml. Bilateral renal venous catheters were introduced via the femoral route under fluoroscopic control and the correct position was ascertained by determination of the PAH extraction.

After completion of this preparatory work, half an hour was allowed for rest. Basal blood samples were then simultaneously taken from the artery and the two renal veins for determination of plasma renin activity (PRA).

In the first part of this study the patient was then tilted 60–70° head-up on a tilt table equipped with bicycle saddle, thereby allowing passive standing position for 10–12 min. A new set of simultaneous blood samples was taken at the end of the tilting procedure.

In the other part of the study graded, slow hemor

Table III. *Data on non-operated patients (renovascular lesions, group 1 c)*

Abbreviations as in Table II

Pat. no.	Procedure	BP (mmHg)	HR (beats/min)	Estimated RPF (ml/min) R	L	PRA (ng angiotensin/100 ml) A	RRV	LRV	$(V-A)_{PRA}$ diff. (ng angiotensin/100 ml) R	L	RSR (ng angiotensin/min) R	L	Renin PRA ratio
4	Basal	198/179	76	250	250	279	310	307	31	28	78	70	0.99
	Tilt	160/120	115			507	661	1 46	154	739	385	1 848	1.89
14	Basal	165/95	68	150	150	53	50	37	−3	−16	−5	−24	1.35
	Tilt	132/93	96			97	73	142	−24	45	−36	68	0.51
16	Basal	180/85	60	200	200	58	66	24	8	−34	16	−68	0.36
	Tilt	100/70	72			105	76	65	−29	−40	−58	−80	0.86
18	Basal	190/108	—	120	240	155	163	153	8	−2	10	−6	1.07
	Tilt	140/90	—			181	191	321	10	140	12	392	0.60
21	Basal	160/100	—	250	250	85	63	74	−22	−11	−55	−28	0.85
	Tilt	110/80	—			101	80	74	21	−27	53	−68	1.08
22	Basal	205/105	62	125	125	48	71	61	23	13	29	16	1.16
	Tilt	125/90	77			90	172	183	82	93	103	116	0.94
23	Basal	190/115	72	160	240	55	130	84	75	29	130	70	1.55
	Tilt	160/110	96			90	198	112	108	22	173	53	1.77
105	Basal	166/85	56	200	200	92	72	96	−20	4	−40	8	0.75
	Hemorrhage	135/76	57			100	100	79	0	−1	0	−42	1.27

rhage was used as a stimulatory procedure, a total of approximately 500 ml blood being withdrawn during 20–30 min, including the two sets of blood samples as described above.

Intraarterial BP was measured with an inductance transducer (Elema) and heart rate (HR) was counted on the ECG recordings.

METHODS

PRA was estimated by the method of Boucher et al. (2, 3). Disodium EDTA was used as anticoagulant, with a 3-hour incubation period. Angiotensin was estimated by the BP method in a 4-point assay on pentolinium-blocked nephrectomized rats under barbiturate anesthesia with electromanometric recording of the mean carotid pressure. Synthetic val-5-angiotensin (Ciba) was used as standard. The error of a single determination is ±20%, and the recovery of added angiotensin to the extraction procedure amounts to 75% in our laboratory. The commonly observed range of PRA may be given as 100–400 ng angiotensin/100 ml. An attempt to estimate the renal plasma flow (RPF) for individual kidneys were made on the basis of total renal clearance of PAH and isotope renography performed a few days prior to the present investigation. These methods have been described previously (1, 10). The estimated RSR for each kidney was calculated as

$$RSR = RPF \cdot (V-A)_{PRA}$$

where RPF is the renal plasma flow and $(V-A)_{PRA}$ the veno-arterial difference of PRA over the kidney.

At a PRA of 400 ng angiotensin/100 ml, a RPF of 300 ml/min and a veno-arterial difference of renin activity over the kidney of 20%, the RSR may be calculated as 240 ng angiotensin/min. A value of up to 300 ng angiotensin/min is therefore used as an estimate of the highest RSR which may be observed in normal kidneys.

RESULTS

The condensed results for the operated patients are given in Table II and for the non-operated patients in Tables III and IV. For operated patients the estimated renin secretion for the two kidneys during supine rest and during stimulation are shown in Fig. 1.

The mean arterial PRA was clearly higher in the successfully operated group (1 a) than in the others, although within normal limits (mean value 323 ng angiotensin/100 ml/3 h). It was rather the other groups that presented with low renin activity values (mean values 129, 75 and 85 ng angiotensin/100 ml/3 h). During stimulation this difference between group 1 a and the other groups was accentuated as 4 of 5 patients in group 1 a increased their arterial PRA substantially while patients in the other groups did not, with two exceptions (nos. 4 and 14).

In successfully operated patients (group 1 a) the estimated renin secretion from the involved kidneys was more than 300 ng angiotensin/min during supine rest in only one (no. 9). In this patient an equally increased secretion was measured on the contralateral side. In the other pa-

Table IV *Data on non-operated patients (parenchymal renal disease)*

Abbreviations as in Table II

Pat. no.	Procedure	BP (mmHg)	HR (beats/min)	Estimated PRF (ml/min) R	Estimated PRF (ml/min) L	PRA (ng angiotensin/100 ml) A	PRA RRV	PRA LRV	$(V-A)_{PRA}$ diff (ng angiotensin/100 ml) R	$(V-A)_{PRA}$ diff L	RSR (ng angiotensin/min) R	RSR L	Renal vein PRA ratio
Group 2													
10	Basal	200/114	96	40	360	120	183	137	63	17	25	61	1.34
	Tilt	154/108	130			126	179	175	53	49	21	176	1.02
13	Basal	135/90	76	40	360	54	104	74	50	20	20	72	1.41
	Tilt	96/80	90			73	95	90	22	17	9	61	1.05
17	Basal	140/82	96	40	360	18	43	87	25	69	10	248	0.49
	Tilt	100/60	88			8	29	39	21	31	8	112	0.74
27	Basal	206/118	68	80	320	83	100	72	17	−11	14	−35	1.39
	Tilt	150/110	80			82	91	75	9	−7	7	−22	1.21
106	Basal	169/105	66	100	400	60	120	77	60	68	60	68	1.56
	Hemorrhage	164/101	56			62	85	91	23	−29	23	116	0.93
107	Basal	214/96	75	40	360	31	48	<100	17	—	7	0	0.48
	Hemorrhage	193/98	78			39	32	37	−7	−2	−3	−7	0.86
110	Basal	161/84	59	360	40	108	101	60	−7	−48	−25	−19	0.99
	Hemorrhage	153/99	57			43	163	169	120	126	432	50	1.04
Group 2b													
24	Basal	184/94	62	250	250	195	180	188	−15	−7	−38	−18	0.96
	Tilt	165/110	74			492	998	1 143	506	651	1 265	1 628	0.87

tients the contralateral secretion was nil in one (no. 2) and showed negative values in three (nos. 8, 101, 108). This was due to the negative veno-arterial renin difference, i.e. the renin activity was higher in the arterial than in the renal venous plasma. During stimulation the renin secretion was increased in all involved kidneys. This increase was more pronounced for orthostatic stimulation (nos. 2, 8, 9) than for slow hemorrhage (nos. 101, 108). The uninvolved kidneys were unresponsive and continued to show negative values in three of the fi[illegible] patients.

The ratio of renal venous renin activity was less than 2.0 in two patients (nos. [illegible] and 9) during supine rest. When stimulation was applied, all ratios were more than 2.0.

In unsuccessfully operated patients (group 1 *b*) the RSR during supine rest was low on both sides and upon stimulation no responsive kidneys were found. The ratios were all low except in one patient (no. 104).

In non-operated patients with renovascular lesions (group 1 *c*) no patient showed increased renin secretion during supine rest. Upon stimulation one patient (no. 4) showed increased secretion. The ratio in this patient was 0.99 during supine rest and 1.89 when stimulated. The patient had bilateral fibromuscular dysplasia with lesions on both sides, but she was not operated upon because surgical correction was considered impossible. The ratios in the other patients in this group were less than 1.6.

In unilateral renal disease (groups [illegible] *a* and *b*) no kidneys showed increased secretion rate during supine rest. Upon stimulation the single case presenting with hydronephrosis (no. [illegible]4) showed an increased secretion, but of equal magnitude, on the two sides. The patients with unilateral contracted kidney showed no increased secretion on stimulation. The ratios in these patients were all low (<1.6) both in the basal and in the stimulated situation.

The BP and HR response during stimulation were approximately the same in all groups, as shown in the Tables.

The previous antihypertensive treatment was apparently not critical for the response to stimulation, as responsive and unresponsive patients had very much the same antihypertensive treatment.

DISCUSSION

In the present series the estimated renin secretion during supine rest was increased in hardly any

Table III *Data on non-operated patients (renovascular lesions, group 1 c)*

Abbreviations as in Table II

Pat. no.	Procedure	BP (mmHg)	HR (*beats/min*)	Estimated RPF (ml/min) R	Estimated RPF (ml/min) L	PRA (ng angiotensin/100 ml) A	PRA RRV	PRA LRV	$(V-A)_{PRA}$ diff. (ng angiotensin/100 ml) R	$(V-A)_{PRA}$ diff. L	RSR (ng angiotensin/min) R	RSR L	Renal cir PRA ratio
4	Basal	198/120	76	250	250	279	310	307	31	48	72	70	0.99
	Tilt	160/120	115			507	661	1 246	154	739	385	1 048	1.89
14	Basal	165/95	68	130	150	33	30	37	−3	−16	−3	−24	1.35
	Tilt	13./93	96			97	73	142	−24	45	−36	68	0.51
16	Basal	180/85	60	200	200	58	66	24	8	−34	16	−68	0.36
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	Tilt	125/90	72			90	172	183	82	93	103	116	0.94
23	Basal	190/115	72	160	240	55	130	85	75	29	120	70	1.55
	Tilt	160/110	96			90	198	112	108	22	173	53	1.77
103	Basal	166/85	56	200	200	92	72	96	−20	4	−40	8	0.75
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where RPF is the renal plasma flow and $(V-A)_{PRA}$ the veno-arterial difference of PRA over the kidney.

At PRA of 400 ng angiotensin/100 ml, a RPF of 300 ml/min and veno-arterial difference of renin activity over the kidney of 20%, the RSR may be calculated as 240 ng angiotensin/min. A value of up to 300 ng angiotensin/min is therefore used as an estimate of the highest RSR which may be observed in normal kidneys.

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The mean arterial PRA was clearly higher in *the successfully operated group (1 a) than in the* others, although within normal limits (mean value 323 ng angiotensin/100 ml/3 h). It was rather the other groups that presented with low renin activity values (mean values 129, 75 and 85 ng angiotensin/100 ml/3 h). During stimulation this difference between group 1 *a* and the other groups was accentuated, as 4 of 5 patients in group 1 *a* increased their arterial PRA substantially while patients in the other groups did not, with two exceptions (nos. 4 and 24).

In successfully operated patients (group 1 *a*) the estimated renin secretion from the *involved* kidneys was more than 300 ng angiotensin/min during supine rest in only one (no. 9). In this patient an equally increased secretion was measured on the contralateral side. In the other pa-

emphasis placed on an estimation of the RSR. The ratio is of doubtful physiological significance, while the RSR may at least offer some insight into the dynamics of the system both qualitatively and quantitatively.

The estimation of the RSR raises some methodological problems, however. We strongly advocate simultaneous blood sampling from an artery and the two renal veins, as it is well known that the renin activity may change within a short period, especially when stimulating procedures are attempted (10, 11). The simultaneous measurement of the renal plasma flow in the individual kidney by means of e.g. bilateral renal clearance would make the investigation a quite formidable procedure. We have therefore estimated the individual flow rate, based on previous investigations of the total renal clearance and isotope renography. This may be quite sufficient for use in the basal state, but may not hold true when stimulation is applied. The assumption is therefore made that the flow is changed to the same extent in the two kidneys and will therefore not upset the relative contribution of each kidney. The stimulation procedures we used, upright posture and slow bleeding, both tend to decrease flow and, therefore, the calculated values for RSR in the stimulated state will be overestimated by at least some 10–20%.

Usually attention is directed towards the involved kidney in the diagnostic work-up of patients with renovascular hypertension. The function of the contralateral, uninvolved kidney may be equally important to assess. In the present series 3 out of 5 successfully operated patients demonstrated a "renin uptake" of the uninvolved kidney, i.e. the arterial renin activity was higher than the renal venous renin activity. To a certain extent this may be due to a chance variation. Scattered throughout in many series one finds observations of this type, although rarely commented upon. Special emphasis may be attached to the reports of Kaneko et al. (10), Strong et al. (15) and Bozovic et al. (5), showing 3/7, 20/28 and 4/5 such cases respectively. Kaneko et al. suggested that removal of renin in the renal lymphatics may play a role, Strong et al. considered the phenomenon as "statistical variation", and Bozovic et al. suggested the presence of renin inhibitors or vasodilating substances in the renal venous plasma. Gunnells et al. (8) also observed this frequent "renin uptake" of the uninvolved kidney but did not comment upon their findings. Significantly, Kaneko et al. observed no negative veno-arterial differences in 13 normotensive control subjects studied both during supine rest and after stimulation of the renin secretion by BP reduction. In our series, where two full sets of samples were obtained, so minimizing a statistical influence, we feel inclined to ascribe to the finding of one renin-secreting and one "renin-consuming" kidney a certain significance in the diagnostic decisions. This "renin consumption" of the uninvolved kidney may be an expression of an antihypertensive function of that kidney possibly related to the renin inhibitor system of Smeby and Bumpus (14).

In the non-operated part of the material no conclusion as to the predictive value of the lack of responsiveness of the kidneys can be drawn. Only one patient showed a substantial response of one kidney, but a corrective operation was considered technically impossible. On the other hand it may be concluded that, in the case presenting with hypertension and unilateral contracted kidney, it must be unusual to find an abnormally elevated RSR in the supine resting as well as in the stimulated state. Similar experience has been reported by Gunnells et al. (8), Fitz (6) and Hollenberg et al. (9).

Till now we have neither observed a patient with unresponsive renin secretion of the involved kidney where surgery has cured the hypertension, nor a patient with responsive renin secretion where surgery has failed. The former question is by far the most important. For renovascular lesions this observation may be true, but for parenchymal renal disease we do not feel nearly as sure. Further studies are required to clarify this point. The latter question may never be adequately settled, because it is greatly dependent on the skill and success of the surgeon and the progressive nature of the disease. Our present experience, however, points in the direction that patients in whom the renin secretion is responsive to stimulation have a fair chance to benefit from surgery.

The antihypertensive treatment was withheld only on the day of investigation. This may explain the rather low arterial renin activity levels in all groups of patients during supine rest. During stimulation, however, the patients with sig-

nificant renovascular lesions (group 1 a,) increased their arterial PRA far more than the other groups. Interestingly no systematic error seems to have been introduced, as responsive and unresponsive patients had very similar treatment. The practical importance of this finding is great indeed, as we are very reluctant to withhold treatment for longer periods in severely hypertensive patients. Often two or three weeks without treatment are advocated previous to the investigation, but this does not seem necessary.

Our experience of the estimation of RSR of individual kidneys in hypertensive patients, as discussed in this paper is encouraging. Much more work is required, however before the perfect examination procedure can be devised. At least in part our stimulation procedure is aimed at stimulation through reduction of the BP This may be potentially dangerous in these often severely ill patients, but no serious side-effects were observed in the present series. Also it may be hoped that the advent of more specific methods (7) than the presently used bioassay technique for PRA estimations will help to clarify further the role and function of the renin angiotensin system in human renovascular hypertension.

ACKNOWLEDGEMENT

This work as supported by the Swedish Medical Research Council (Project no. 19X-2451).

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Acta med. scand. Vol. 193 pp. 407–410, 1973

THE EFFECT OF DIFFERENT DIURETICS ON ELEVATED BLOOD PRESSURE AND SERUM POTASSIUM

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Abstract. A *material of 22 patients with essential benign* hypertension has been submitted to a double-blind investigation, in which each patient received hydrochlorothiazide 25, 50, 75 and 100 mg daily, clopamide 10 and 20 mg daily, cyclothiazide 2 mg daily and a placebo. *Each of these dosages was administered for at least* 6 weeks. The mean blood pressure and serum electrolyte values during administration of the placebo and the changes in these values during treatment are accounted for. The dose effect curve of hydrochlorothiazide is characterized by a considerable *fall in BP following* administration of the lowest dose. When the dosage is increased, the fall in BP is linear and relatively slight. As the reduction in serum potassium during treatment showed the same pattern, it could be demonstrated that, *in the material as* a whole, *and within the range investigated*, there was a direct proportionality between the fall in BP and the reduction in serum potassium. Clopamide and cyclothiazide in equipotent doses resulted in the same degree of hypokalaemia as did hydrochlorothiazide.

In the treatment of hypertension, diuretics (thiazides and related compounds) are of particular importance. In milder cases a sufficient reduction of blood pressure can often be achieved, in the more severe cases *the diuretics will constitute a* valuable supplement to the more specific and *potent antihypertensive drugs.*

An important side-effect of the diuretics mentioned is the accompanying *decrease in serum* potassium. To obtain information about the optimal intensity of treatment, we have studied the effect of various doses of one of the most commonly used thiazides (hydrochlorothiazide) both on blood pressure and serum potassium. Furthermore two fairly new diuretics, clopamide and cyclothiazide, have been included in the trial, especially in order to study the hypokalaemic effect of these drugs as compared to that of hydrochlorothiazide.

MATERIAL AND METHODS

Thirty-one patients with hypertension requiring treatment were studied. No special selection of the patients was made apart from the fact that the hypertension should not be so severe that temporary withdrawal of antihypertensive treatment (during placebo intake) could be dangerous. In the course of the year prior to this investigation all patients were hospitalized for clarification of diagnosis and aetiology or for a brief check-up.

Three drop-outs occurred during the relatively long trial (of more than one year) for the following reasons.

One patient exhibited during the investigation period a highly elevated arterial BP which needed immediate treatment. For another patient the frequent visits to the clinic would have had too severe financial consequences. The third patient was excluded from the material because her BP was affected by an operation.

Six patients were excluded for *failing to keep* to the prescribed intake of drugs (see below).

Consequently the final material consists of 22 patients (11 women aged 40–66 and 11 men aged 43–75). In all patients, according to our results, the hypertension must be characterized as essential.

Programme of investigation

The drugs studied were hydrochlorothiazide (Esidrex®), clopamide (Adurix®, Brinaldix®) and cyclothiazide (Doburil®). The dose response curve for hydrochlorothiazide was constructed by using the doses 25, 50, 75 and 100 mg/day. After study of the available literature, the doses 10 and 20 mg clopamide (2, 4, 7, 11) and 2 mg cyclothiazide (8, 9, 10) were chosen.

The investigation was arranged as a double-blind trial, each patient serving as his own control.

Each patient received, according to a previously planned scheme, the following medication: hydrochlorothiazide in doses of 25, 50, 75, and 100 mg daily, clopamide in *doses of 10 and 20 mg daily*, cyclothiazide 2 mg *daily* and a placebo. The sequence in which the patients took the drugs was planned in such a manner that all possible changes from one medication to another occurred.

The duration of treatment in each of the eight periods was a *minimum of 6 weeks*. The patients took two tablets twice a day during the whole investigation, thus receiving the previously mentioned dosages of the four different

Table I *Data during placebo administration*

	Mean	Range	S.D.
Mean BP (mmHg)	127.34	112.9–150.3	10.33
Serum potassium (mEq/l)	4.12	3.35–5.15	0.48
Serum chloride (mEq/l)	104.20	100.5–110.5	2.33
Serum bicarbonate (mEq/l)	25.59	22.5–27.5	1.36
Serum creatinine (mEq/l)	1.11	0.85–1.43	0.186

compounds. The patients returned the unused tablets at the end of each period, thus making it possible to check whether they had conformed to the prescribed dosage. As previously mentioned, six patients had to be excluded as they had taken less than 80% of the tablets in at least one of the eight periods.

During each period BP was measured after 3, 5 and 6 weeks, 3 times in the supine position after 10 min rest. As no systematic variation was observed between the measurements during the 3 visits in any period, the average of these 9 measurements was considered representative for a given medication.

The mean BP was used and calculated as the diastolic BP + 1/3 of the BP amplitude.

Prior to the end of each period—5 and 6 weeks after the beginning of each medication—blood samples were taken for determination of serum potassium, chloride, bicarbonate and creatinine. The average of two corresponding serum electrolyte or serum creatinine values was used in the calculations.

Statistical methods

Unless otherwise stated, changes in the mean BP serum electrolytes and serum creatinine seen during the seven different periods are related to the values obtained during the administration of placebo. The importance to be attached to these changes is evaluated by the *t*-test for pair differences. All other differences are evaluated using Wilcoxon test for pair differences. Other statistical methods appear from the text.

RESULTS

The most important results are presented in Tables I and II.

After administration of 25 mg hydrochlorothiazide the mean BP fell 11 mmHg ($p < 0.001$). A further reduction of about [illegible] mmHg was noticed for every 25 mg increase in dosage. The fall in mean BP obtained by increasing the dosage of hydrochlorothiazide from 25 to 75 mg was significant ($p < 0.05$).

Clopamide in doses of 10 and 20 mg daily caused a decrease in mean BP of about 16 mmHg. This fall in BP does not differ from that obtained with 75 and 100 mg hydrochlorothiazide daily.

Cyclothiazide 2 mg daily only resulted in a moderate, but still significant decrease in mean BP ($p < 0.001$).

A dosage of 25 mg hydrochlorothiazide caused a decrease in serum potassium of 0.46 mEq/l ($p < 0.001$). Serum potassium was reduced by slightly more than 0.1 mEq/l for each increase of [illegible] mg in the dosage.

The reduction in serum potassium after administration of clopamide in the two dosages corresponded to the reduction seen after administration of 75 mg hydrochlorothiazide—slightly more after 20 than after 10 mg clopamide daily ($p < 0.05$).

Cyclothiazide 2 mg daily produced a small, but significant reduction in the serum potassium ($p < 0.001$).

Serum chloride was reduced after administration of the various diuretics. The reduction was seen already after administration of 25 mg hydrochlorothiazide daily (3.18 mEq/l) and was significant ($p < 0.001$). Hydrochlorothiazide 25 mg daily caused an increase in bicarbonte of 1.43 mEq/l ($p < 0.01$). The three other doses resulted in greater increases in bicarbonate.

No changes in serum creatinine occurred during administration of the various drugs.

Table II *Changes in mean BP serum electrolytes and serum creatinine for the different treatment periods*

	Daily dose (mg)	Decrease in Mean BP ($\bar{X}$)	(S.D.)	Serum potassium ($\bar{X}$)	(S.D.)	Serum chloride ($\bar{X}$)	(S.D.)	Increase in Serum bicarbonate ($\bar{X}$)	(S.D.)	Serum creatinine ($\bar{X}$)	(S.D.)
Hydrochlorothiazide	25	11.07	11.21	0.46	0.25	3.18	3.40	1.43	2.28	0.034	0.105
	50	13.08	10.38	0.58	0.32	3.61	3.15	3.00	2.04	−0.009	0.088
	75	15.01	10.04	0.68	0.53	4.91	3.25	2.48	2.39	−0.009	0.093
	100	16.90	9.[illegible]	0.81	0.62	5.66	3.93	3.05	3.07	0.023	0.123
Clopamide	10	15.79	7.93	0.63	0.39	6.00	3.09	2.61	2.10	−0.009	0.117
	20	16.12	10.96	0.73	0.36	7.23	2.94	3.34	1.83	0.023	0.087
Cyclothiazide	2	7.77	8.90	0.38	0.40	3.14	2.77	2.05	1.57	−0.005	0.126

Fig. 1 shows the connection between the patients' BP during administration of placebo and the fall in BP obtained by administration of the four different dosages of hydrochlorothiazide. This Figure illustrates the previously mentioned phenomenon, that the fall in BP increased after administration of increasing doses of hydrochlorothiazide. It can also be seen that, as the regression lines are parallel, the increased effect obtained by increasing the dosage applies equally to the low as to the high BP measured during placebo administration. Finally it can be seen that the higher the placebo BP the greater the fall in BP: this applies to all four dosages. This means that a normal BP will not—or at least only slightly—be affected by the treatment.

The fact that the fall in BP and the reduction in serum potassium were quite pronounced after administration of 25 mg hydrochlorothiazide, following which there was only a slight additional fall with increasing dosage, drew attention to the desirability of Fig. 2. This Figure illustrates *the connection between the fall in BP and the* reduction in the level of serum potassium, as found in the whole material.

There was no clear connection between these two parameters in the individual patients, but the material as a whole showed direct proportionality between fall in BP and reduction in serum potassium.

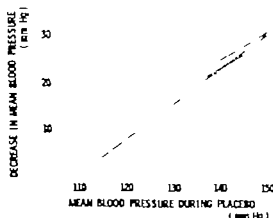


Fig. 1. Regression lines of decrease in mean BP during treatment with the four doses of hydrochlorothiazide on BP during placebo. The equations (with correlation coefficients within parentheses) are:

25 mg $y = 0.73x - 81.63$ ($r = 0.67$, p 0.001)
50 mg y 0.76$x - 84.09$ ($r = 0.75$, $p < 0.001$)
75 mg $y = 0.6$[illegible]$x - 64.22$ ($r = 0.64$, p 0.01)
—— 100 mg $y = 0.61x - 60.82$ ($r = 0.66$, $p < 0.001$).

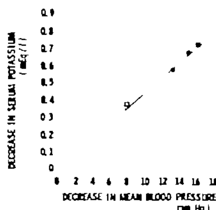


Fig. 2. Corresponding values for decrease in mean BP and decrease in serum potassium during treatment with the four doses of hydrochlorothiazide (○), clopamide in the doses 10 and 20 mg/day (◻ and ■ respectively) and cyclothiazide 2 mg/day (□). Each point represents an average of 22 corresponding values.

As the interdependent values for the fall in BP and the reduction in the serum potassium during administration of clopamide and cyclothiazide lie on a line that can be drawn through the four points of hydrochlorothiazide (Fig. 2), it may be concluded that these two diuretics in equipotent doses result in the same degree of hypokalaemia *as does hydrochlorothiazide.*

No side-effects caused the drugs to be withdrawn from any of the patients during the investigation. About a third of the patients complained of slight gastrointestinal or neuromuscular side-effects after administration of 75 and 100 mg hydrochlorothiazide and 10 and 20 mg clopamide daily. A few suffered from palpitations, tinnitus, a tendency to fainting, dizziness or pronounced tiredness. These side-effects were hardly ever seen after administration of 2 mg cyclothiazide or hydrochlorothiazide in the two lowest doses.

DISCUSSION

The planning and a number of the results in the present investigation are very similar to the long-term study carried out by Cranston et al. (3). These investigators studied the effect on increased BP of the administration of cyclopenthiazide, chlorthalidone and bendrofluazide—each of these three diuretics in three different dosages.

The present investigation has demonstrated that a very considerable fall in BP occurs after ad-

ministration of the lowest doses. Only a very slight additional decrease in BP occurs with a further increase of the dosage. The fact that the same dose of a thiazide results in a greater fall in BP if the hypertension is severe than if it is slight (Fig. 1) has also been demonstrated by previous investigators (3 5).

However the close connection between reduction in BP and decrease in serum potassium has not, to our knowledge, been described previously. The investigation of Cranston et al. (3) is not particularly suitable for illustrating this question, as the patients in their material received a standard supplement of potassium.

The straight line (Fig. 2) that corresponds best to the seven average values found has the equation $y = 0.044x + 0.0096$. This means that, in the range observed, there is a direct proportionality between the fall in BP and the reduction in serum potassium. No corresponding regular connection exists between the fall in BP and the remaining electrolyte changes measured.

The proportionality found between the fall in BP and the reduction in serum potassium has a certain amount of practical interest, but it is difficult at the present time to determine whether this intimate connection can add to our knowledge of the BP-reducing effect of thiazides. It is possible that this effect is so bound to a certain degree of hypokalaemia that an attempt to normalize the hypokalaemia by giving a supplement of potassium will counteract the fall in BP (1 5 6).

The results of the present investigation do not provide an answer to the question of what dosage of thiazides should be used in the treatment of hypertension. An increased effect is obtained by increasing the dosage, but at the same time a progressive reduction in serum potassium and more marked side-effects occur. It is worth noting that even a relatively small dose (e.g. 25 mg hydrochlorothiazide/day) produces a considerable fall in the mean BP. An increase in effect of only 18% is obtained by doubling the dosage.

The various thiazides and thiazide-like drugs differ mainly in the duration of their effect. It has not been possible, to date, to demonstrate that thiazides differ in their ability to produce hypokalaemia.

Cyclothiazide, which is in actual fact a thiazide drug, and clopamide, which like chlorthalidone (Hygroton®), belong to the thiazide-like diuretics, have in the present investigation been seen to produce the same degree of hypokalaemia as hydrochlorothiazide in doses having the same antihypertensive effect.

Clopamide in doses of 10 and 20 mg daily resulted in the same fall in BP. However 20 mg clopamide caused a greater reduction in serum potassium. Consequently a daily dose of 10 rather than 20 mg clopamide must be recommended in the treatment of hypertension.

Cyclothiazide at a dosage level of 2 mg daily was seen to have only a moderate effect on the increased BP—an effect that corresponded to that of almost 25 mg hydrochlorothiazide. This dosage turned out to be smaller than we anticipated. Nineteen of the 24 patients have continued the treatment of their hypertension with 5 mg cyclothiazide daily after the completion of the present investigation. At this dosage level we have obtained a mean fall in BP that corresponds to that obtained after administration of 50–100 mg hydrochlorothiazide. A dosage of 4–5 mg daily would probably be the most advantageous level for cyclothiazide when used in the treatment of hypertension.

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Acta med. scand. Vol. 193 pp. 411–418, 1973

THE EFFECT OF ALPRENOLOL ON HEMODYNAMICS IN ANGINA PECTORIS

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Abstract. Twelve men between 41 and 67 years of age were admitted for preoperative evaluation of angina pectoris. The diagnosis was confirmed by history, ECG at rest and exercise, and coronary arteriograms. Right heart and transseptal left heart catheterization were performed. Cardiac output and intracardiac pressures were determined at rest and during exercise with a load which caused angina pectoris. A β-adrenergic blocking agent, alprenolol, was then injected intravenously, whereupon the studies at rest and exercise were repeated. The racemic form was given to 8 cases (0.1 mg/kg) and the levo form to 4 cases (0.05 mg/kg). During the first exercise period a pressure rise in the left atrium was found in all cases but one. Alprenolol caused few changes at rest. During the second exercise, with the same load, there was less increase in heart rate and aortic pressure after alprenolol. Four cases could perform the same work after alprenolol with no or with less pain than before. This group differed from the remaining 8 cases in having a higher left atrial pressure rise during the first work test, which also caused a higher heart rate in relation to oxygen consumption. This could mean that they were more hyperkinetic. The improved subjective exercise tolerance in this group was accompanied by less increase in heart rate and left atrial pressure than in those with the same degree of pain.

It has been known since the pioneer work by Müller and Rørvik (27) that spontaneous as well as effort-precipitated angina pectoris is accompanied by an increase in pulmonary capillary venous pressure, which can be prevented by nitroglycerine. This can be used for testing antianginal drugs. The author has investigated a series of 12 patients with angina pectoris by means of right *and left heart catheterization in order to study* the potency of the β-adrenergic blocking agent alprenolol (9) to influence the subjective and hemodynamic response to an exercise test.

MATERIAL

Twelve men with angina pectoris were investigated (Table I). They were between 41 and 67 years of age and had suffered from effort angina for between 6 months and 10 years. In 8 cases there was a history of at least one myocardial infarction. There were no signs of valvular heart disease or pulmonary disease.

The ECG at rest was normal in 2 cases, one of whom had a history of myocardial infarction. In one case there were borderline ST-T changes, while the ECG was abnormal in the remaining 9 cases, with LBBB in cases and postinfarction pattern in 4. Abnormal degree and type ("ischemic") of ST depression occurred in all cases during the exercise test, except in one with a postinfarction pattern ECG.

Prior to the hemodynamic study an exercise test was performed in sitting position on an electrically braked bicycle ergometer. ECG was recorded continuously. The patients worked for 6 min on each load. The times when angina pectoris appeared and when the work had to be discontinued are given in Table I. All but 2 cases tolerated a load of 400 kpm min or less before pain appeared. The exercise test was stopped after a further 2–4 min because of pain and or ECG changes.

Coronary arteriograms were recorded by a non-selective method as earlier described (4). The findings were classified from grade 1, no changes or minimal wall irregularities, to grade 5, total occlusion. Pronounced narrowing (grades 3–5) was present in 10 cases. The findings at left ventricular angiography are only given as an estimation of the end-diastolic and end-systolic volumes, and the occurrence of local dyskinesia. Abnormal findings were found in 4 cases. The roentgenological heart volume was determined in sitting position (22). As can be seen in Table I, the heart size was normal in all cases. This is to some extent due to the principles of selection of patients for preoperative evaluation. It may be added that we have now changed to a selective technique for coronary arteriograms.

METHODS

The hemodynamic investigation was performed on the fourth day after admission, in fasting state and in supine

Table I. *Clinical data*

RC, LAD and LC = right, left anterior descending and left circumflex coronary artery: 1 5 = degree of coronary atheromatoses, ED = end-diastolic, ES = end-systolic volume; Dyskin. = local dyskinesia 100 kpm = 16.34 watts

					Exercise test (load and time) when											
					Angina appeared		Work discont.		Heart volume		Coronary angiography			Left ventricular angiography		
Case no.	Age (y)	BSA (m²)	Myoc. infarct. (n)	Duration of ang. pect. (y.)	(kpm/min)	(min)	(kpm/min)	(min)	(ml)	(ml/m²)	RC	LAD	LC	ED	ES	Dyskin.
1	48	1.86	1	2	300	1	300	4	790	425	3	5	5	0	0	0
2	44	1.90	1	2	400	2	600	1.25	760	400	5	3	3	0	+	0
3	57	1.32	2	3	300	3	450	2	600	455	3	2	1	+	+	0
4	41	1.67	0	2	400	2	400	4	600	360	2	3	3	0	0	0
5	60	1.95	1	7	400	3	400	5	1 010	520	5	4	3	+	+	0
6	64	1.78	1	6	600	0.5	600	2.5	660	370	5	2	2	0	0	0
7	54	1.83	2 ?	6	600	2	800	1	750	410	5	3	3	0	+	+
8	67	1.73	0	10	400	4	400	4.5	710	410	Arrhythmia			Arrhythmia		
9	51	2.10	0	1/2	400	0	500	2	870	415	2	2	1	0	0	0
10	56	1.87	1	7	200	3	200	5.5	840	450	5	4	2	0	0	?
11	58	2.26	0	4	200	6	200	6	1 030	450	2	3	2	0	0	0
12	62	1.95	1	4	100	2	100	6	800	410	5	2	2	0	0	0

position. Catheters were introduced to the aortic arch, pulmonary artery and, by transseptal puncture, the left atrium (LA). After 10 min rest cardiac output was determined by Fick's direct method, with simultaneous measurement of heart rate and intracardiac pressures. Expired air was collected during 10 min. The error of a single determination of cardiac output by the Fick technique in this laboratory is 6.1% (20).

The patient then worked on a bicycle ergometer. After 2–3 min of exercise the cardiac output was again measured during 3 min. The total exercise period was between 4 and 6 min, depending on the degree of pain provoked. After 10 min rest alprenolol was injected via the right heart catheter into the pulmonary artery. A dose of 0.1 mg/kg b.wt. was given at a rate of 1 mg/min. In 4 cases the levo-isomer was given, in half dose, which produces the same degree of β-blockade as the dose of the racemate used (1). The degree of β-blockade obtained was not tested (for ref. see 1, 14). After 10 min rest the procedure was repeated. Thus the rest period between the exercise periods was about 35 min. Finally coronary arteriography and left ventricular angiography were performed.

There were no immediate complications, and no late which could be ascribed to the hemodynamic investigation. In case 5 myocardial infarction occurred 3 days after the investigation and the patient died after further weeks. In case 9 the angina pectoris was more protracted after the second work test (with alprenolol), necessitating nitroglycerine sublingually 10 min after the end of the work. The angiographic investigation had to be postponed till the next day.

The results are analysed with Student's *t*-test, and the probability of significance of changes relates to comparison of paired samples. The statistical evaluation was made by dr G. Nyberg.

RESULTS

Individual data are presented in Table II. Mean values are found in Table III for all 12 cases (group A) and for those 4 cases (nos. 1 4 10 12) who had less anginal pain at the second work test (group B) and for those with approximately the same degree of pain at the second test (group C). The analysis of changes from rest to exercise, with and without alprenolol, are given in Table IV. Pressure changes in LA are illustrated in Fig. 1. The product of heart rate and systolic aortic

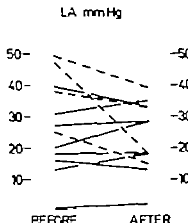


Fig. 1. LA mean pressure during exercise, before and after alprenolol. Cases with less angina pectoris after alprenolol.

Table II. *Hemodynamic findings*

V_{O_2} = oxygen consumption (ml/min), CO = cardiac output (l/min), CI = cardiac index (CO m² BSA), SV = stroke volume (ml), LA = left atrium, PA = pulmonary artery PRP = the product of heart rate and systolic aortic pressure, L = the levo-isomer of alprenolol

								Pressures (mmHg)					
								PA		Aorta			
Case no.	Work load and time	Heart rate	V_{O_2}	CO	CI	SV	LA (mean)	(syst./diast.)	(mean)	(syst./diast.)	(mean)	PRP	Severity of ang. pect.
1	Rest	65	236	5.0	2.7	77	1	13/4	7	122/72	85	7 930	
	200 kpm 5	104	301	4.9	2.6	47	38	55/30	43	152/97	124	15 808	Moderate
	Rest, alpren. 8 mg	64	236	4.4	2.4	69	1	10/5	7	108/64	85	6 912	
	200 kpm 6	89	600	6.0	3.2	67	33	46/32	38	158/98	122	14 062	Slight
2	Rest	59	215	4.9	2.5	83	3	13/5	8	145/90	120	8 555	
	350 kpm 4½	91	693	7.5	3.9	82	20	40/20	25	190/114	140	17 290	Moderate
	Rest, alpren. 8 mg	57	253	5.4	2.8	95	4	17/7	11	148/90	108	8 436	
	350 kpm 4½	90	856	8.2	4.2	91	28	42/26	32	168/108	132	15 120	Moderate
3	Rest	78	222	5.0	3.0	64	4	17/5	10	130/70	86	10 140	
	300 kpm 6	107	833	8.3	4.9	77	13	37/17	25	176/84	130	18 832	Moderate
	Rest, alpren. 6 mg	68	238	4.4	2.6	65	9	22/8	13	150/76	102	10 200	
	300 kpm 6	95	921	8.1	4.8	85	18	39/18	28	156/80	110	14 820	Moderate
4	Rest	91	268	6.1	3.6	67	4	18/10	14	158/96	128	14 378	
	300 kpm 6	142	879	9.9	5.8	70	25	47/33	36	166/108	132	23 572	Moderate
	Rest, alpren. 7 mg	87	257	5.5	3.2	63	2	19/10	14	130/84	112	11 310	
	300 kpm 6	120	849	8.3	4.9	68	15	34/22	28	152/96	120	18 240	None
5	Rest	63	240	5.5	2.8	87	7	28/11	17	160/84	110	10 080	
	300 kpm 6	109	799	7.5	3.8	69	39	72/44	56	160/96	120	17 440	Moderate
	Rest, alpren. 8 mg	61	279	5.4	2.7	89	9	28/10	17	158/80	112	9 638	
	300 kpm 6	97	768	6.5	3.3	67	33	61/34	48	156/90	120	15 132	Moderate
6	Rest	70	180	3.5	1.9	50	3	18/7	12	150/72	96	10 500	
	400 kpm 6	103	1 029	9.9	5.5	96	18	47/18	33	198/90	138	20 394	Moderate
	Rest, alpren. 6½ mg	68	235	4.5	2.5	66	5	25/8	14	148/70	100	10 064	
	400 kpm 6	99	1 022	8.2	4.6	83	18	47/20	32	176/80	118	17 424	Moderate
7	Rest	87	216	4.9	2.8	56	5	20/7	13	120/80	96	10 440	
	600 kpm 6	141	1 187	8.7	4.8	62	27	58/28	43	164/100	126	23 124	Moderate
	Rest, alpren. 7 mg	88	212	4.5	2.5	51	3	20/7	13	110/72	90	9 680	
	600 kpm 6	130	1 218	8.9	4.9	68	28	59/32	45	160/96	124	20 800	Moderate
8	Rest	54	212	3.8	2.2	70	7	18/10	13	117/65	88	6 318	
	300 kpm 5	109	842	7.3	4.2	67	31	48/35	39	145/85	105	15 805	Sever
	Rest, alpren. 7 mg	72	231	4.7	2.7	65	4	17/8	12	140/80	110	10 080	
	300 kpm 4	110	831	7.0	4.1	64	35	55/35	43	145/90	110	15 950	Severe
9	Rest	80	276	5.7	2.8	71	3	23/11	15	120/84	100	9 600	
	400 kpm 6	103	1 040	10.2	4.9	99	16	50/25	35	150/90	120	15 450	Slight
	Rest, alpren. L 5 mg	69	334	6.2	3.0	90	4	20/8	12	116/82	98	8 004	
	400 kpm 6	97	1 114	10.1	4.9	104	13	47/27	33	142/88	110	13 774	Slight
10	Rest	92	284	6.1	3.3	66	7	34/17	23	172/92	125	15 8[illegible]4	
	150 kpm 5	129	619	6.3	3.4	49	—	75/49	58	168/97	138	21 672	Moderate
	Rest, alpren. L 4 mg	77	[illegible]46	5.4	2.9	70	9	30/17	21	160/89	119	12 320	
	150 kpm 6	103	668	6.1	3.3	59	—	64/39	48	180/96	128	18 540	Slight
11	Rest	64	275	6.9	3.1	108	2	19/7	11	118/70	86	7 552	
	150 kpm 6	86	777	11.1	5.0	129	1	24/9	14	138/74	100	11 868	Slight
	Rest, alpren. L 5 mg	59	305	5.7	2.6	97	2	16/6	10	108/66	82	6 372	
	150 kpm 6	75	692	9.7	4.3	129	2	[illegible]3/9	14	111/60	78	8 325	Slight
12	Rest	80	221	3.7	1.9	46	1	37/21	[illegible]6	152/100	122	12 160	
	150 kpm 4	117	672	6.3	3.3	54	47	70/38	54	126/92	106	14 742	Slight
	Rest, alpren. L 4 mg	75	263	4.2	2.2	56	1	15/6	10	148/98	116	11 100	
	150 kpm 4	95	638	5.9	3.1	62	18	60/27	43	138/96	118	13 110	None

pressure (PRP) was used as an index of left ventricular work.

In case 10 the quality of the LA pressure curve at the second exercise test was unacceptable. In this case the diastolic pulmonary artery pressure is used in Fig. 1. A comparison between the LA mean pressure and the pulmonary artery diastolic pressure in this series shows reasonable agreement. No case had angina pectoris in any of the rest periods.

Table III. *Hemodynamic parameters (mean ± S.E.M.)*

R = rest, E = exercise, other abbreviations as in Table II

Group		Heart rate	V_{O_2} (ml/min)	CO (l/min)	SV (ml)	LA (mean)	Pressures (mmHg) PA (mean)	Aorta (syst.)	Aorta (mean)	PRP
A. Total material (n = 12)										
Before alprenolol	R	74 ± 4	237 ± 9	4.8 ± 0.3	70 ± 5	4 ± 1	14 ± 2	139 ± 6	104 ± 5	10 289 ± 794
	E	112 ± 5	824 ± 56	7.7 ± 0.5	75 ± 7	25 ± 4	38 ± 4	161 ± 6	123 ± 4	18 000 ± 1 039
After alprenolol	R	70 ± 3	261 ± 10	4.8 ± 0.3	73 ± 5	4 ± 1	13 ± 1	133 ± 6	103 ± 4	9 510 ± 514
	E	100 ± 4	850 ± 56	7.3 ± 0.5	79 ± 6	22 ± 3	36 ± 3	154 ± 5	116 ± 4	15 441 ± 979
B. Pressure effect (n = 4)										
Before alprenolol	R	82 ± 6	252 ± 14	5.2 ± 0.6	64 ± 7	3 ± 1	18 ± 4	151 ± 11	115 ± 10	12 373 ± 1 721
	E	123 ± 8	668 ± 79	6.9 ± 1.1	55 ± 5	37 ± 6	48 ± 5	153 ± 10	125 ± 7	18 949 ± 2 167
After alprenolol	R	76 ± 5	261 ± 10	4.9 ± 0.3	65 ± 3	3 ± 2	13 ± 3	137 ± 11	108 ± 8	10 411 ± 1 196
	E	102 ± 7	669 ± 55	6.6 ± 0.6	64 ± 2	22 ± 6	39 ± 4	157 ± 9	122 ± 2	15 948 ± 1 402
C. No effect (n = 8)										
Before alprenolol	R	69 ± 4	230 ± 12	4.6 ± 0.3	74 ± 7	4 ± 1	12 ± 1	133 ± 6	98 ± 4	9 148 ± 542
	E	106 ± 6	903 ± 59	8.1 ± 0.6	85 ± 8	21 ± 4	34 ± 5	165 ± 8	122 ± 5	17 525 ± 1 200
After alprenolol	R	68 ± 4	261 ± 15	4.7 ± 0.4	77 ± 6	5 ± 1	13 ± 1	135 ± 7	100 ± 4	9 059 ± 478
	E	99 ± 6	930 ± 65	7.7 ± 0.6	86 ± 8	22 ± 4	34 ± 4	152 ± 7	113 ± 6	15 168 ± 1 242

Findings at rest before alprenolol. Though the mean values (Table III) are within normal limits, it can be seen in Table II that sinus tachycardia and a low cardiac output were found in a few cases. The only significant difference between groups B and C (Table III) was for PRP which was higher in group B ($p < 0.05$).

Findings at work before alprenolol. More or less severe angina pectoris was provoked in all cases. As could be expected, there were significant changes from rest to exercise in all parameters studied with the exception of the stroke volume (Table IV). A comparison between mean changes at exercise in groups B and C shows in group B less increase in systolic aortic pressure ($p < 0.025$), a larger increase in mean LA pressure ($p < 0.05$) and a smaller increase in oxygen consumption ($p < 0.05$).

The best straight regression line for oxygen consumption (V_{O_2}) on heart rate in group B is expressed by the equation

$$V_{O_2} = 8.64 \times \text{heart rate} - 396$$

and for group C

$$V_{O_2} = 7.59 \times \text{heart rate} + 97$$

These lines are different ($p < 0.05$).

Findings at rest after alprenolol. In group A there was a small but significant increase in V_{O_2} ($p < 0.01$) compared to values at rest before alprenolol. There was no change in heart rate. The increase in V_{O_2} was evident in group C ($p < 0.005$) but not significant in group B. In group B only was there a significant decrease in PRP ($p < 0.05$).

There was no significant difference between the mean values in groups B and C. If the mean changes are compared, one finds a decrease in systolic aortic pressure ($p < 0.05$) and PRP ($p < 0.10$) in group B compared with group C, where the pressure rose and PRP was unchanged.

Findings at work after alprenolol. In the total material there were significantly lower values for heart rate ($p < 0.001$), mean aortic pressure ($p < 0.05$) and PRP ($p < 0.001$) after than before alprenolol. In group B there was a lower heart rate ($p < 0.005$), mean pulmonary artery pressure ($p < 0.01$) and PRP ($p < 0.05$). In group C there was also a lower heart rate ($p < 0.01$), mean aortic pressure ($p < 0.05$), systolic aortic pressure ($p < 0.01$) and PRP ($p < 0.005$).

If one compares the mean changes at work before and after β-adrenergic blockade in groups B and C, one finds that in group B there is a larger fall in heart rate ($p < 0.001$), in pulmonary artery mean pressure ($p < 0.005$), in LA mean pressure ($p < 0.01$) and an increase in systolic aortic pres-

Table IV *Changes from rest to exercise before and fter alprenolol (mean ± S.E.M)*
Symbols as in Table II

	Group	Rest to exercise before alpren.	Rest to exercise after alpren.	Rest before/after alpren.	Exercise before/after alpren.
Heart rate	A	−38±3	−30±2	3±3	12±3
	B	−41±3	−26±3	6±3	21±2
	C	−37±5	−31±3	2±3	7±2
$\dot{V}O_2$ (ml/min)	A	−587±59*	−589±58	−24±7	−26±21
	B	−416±76	−428±55	−8±12	−21±32
	C	−673±62	−669±68	−31±7	−28±.8
CO (l/min)	A	−2.9±0.5	−2.5±0.4	0.01±0.2	0.4±0.2
	B	−1.6±0.9	−1 7±0 4	0.4±0.3	0.3±0 6
	C	−3.5±0.5	−3.0±0.4	−0.2±0.2	0.4±0.3
SV (ml)	A	−5±6	−6±4	−3±3	−4±2
	B	9±9	0±4	−1±4	−9±4
	C	−12±7	−9±6	−4±4	−1±3
LA	A	−21±4	−18±3	−1±1	3±3
mean pres.	B	−35±7	−21±6	1±1	15±7
(mmHg)	C	−16±4	−17±4	−1±1	−1±2
PA	A	−24±3 **	23±3	1±1	2±2
mean pres.	B	−30±3	−26±4	5±4	9±1
(mmHg)	C	−21±4	−22±3	0±1	−1±2
Aortic	A	−22±7	18±6	3±4	8±4
syst. pres.	B	−2±12	−21±12	14.5±5	−4±6
(mmHg)	C	−33±6	−17±6	−2±5	13±4
Aortic	A	−20±5	−13±4	1±3	7±3
mean pres.	B	−10±11	−14±8	7±3	3±6
(mmHg)	C	−25±5	−13±4	−3±4	10±4
PRP	A	−7 710±787	−5 932±703	780±519	2 558±400*
	B	−6 376±1 440*	−5 578±1 206	2 163±655	2 961±841
	C	−8 377±909*	−6 109±917	89±583	2 357±449**

$-p<0.05$, $-p<0.01$ $-p<0.001$

sure ($p<0.05$) There was no significant dif ference between the changes in PRP

In cases 1 4 10 and 12 the pain was reported to be clearly less severe at the second work load. In case 8 the pain was more persistent after the second work load. It began after 3 1/ min of work in both tests. After the first exercise the pain disappeared spontaneously after 8 min rest. After the second exercise the pain was still significant after 10 min rest, when nitroglycerine was given. In case 2 the pain appeared after 3 / min work in the first test, but already after 2 min work in the second.

DISCUSSION

In order to a oid ventricular arrhythmias during exercise as well as during contrast injection, the transseptal catheter was not placed in the left ventricle. The LA mean pressure was used as a more direct measure of left ventricular filling pressure than pulmonary capillary enous or pulmonary artery diastolic pressure The LA mean pressure should be more reliable, especially when the pulmonary vascular resistance is unknown, and at higher heart rates.

At rest there were no important differences between the patients who benefited from alprenolol (group B) and those who did not (group C) This is alid for hemodynamic parameters studied and also for the severity of the coronary disease, though the groups are small and unequal in size. The mean ge in group B was 51 8 years and in group C 56 9 years; this difference is not significant.

In the second rest period, after alprenolol, the oxygen consumption was higher in the whole group and this was due to a significant increase in group C. Between these two periods not only had alprenolol been given but exercise causing angina pectoris had been performed. As could be expected, the load in supine work had to be

lower than in sitting position (24). Although the load was only slight to moderate angina was precipitated. After cessation of the first work period there was a rapid normalization of an increased LA pressure, as a rule during the first minute of rest. When cardiac pressures and output were determined during the second rest period, about 25 min after the first work load, similar values were found. For this reason the remaining oxygen debt or increased respiratory work due to left ventricular failure can be excluded as the cause of the increased V_{O_2}. Individual deviations from steady state after exercise are unknown in respect of degree and direction and might have an impact on the results.

Studies on the effects of repeated exercise periods on hemodynamics at rest have revealed an insignificant decrease in V_{O_2} (11, 26).

The slight decrease in PRP in group B at rest after alprenolol was not seen in group C. This might reflex differences in persistent muscular tension and sympathetic overdrive due to apprehension after the first provoked anginal attack. If so, it probably depends more on constitutional factors than on the severity of the pain, though this is difficult to assess. It has been shown that patients with angina pectoris as a group may have higher sympathetic activity than a control group (16, 28). There may also be differences in this respect within groups with angina pectoris, which could explain variations in therapeutic activity of β-adrenergic blocking agents.

A constant individual relationship has been reported between the appearance of angina pectoris and left ventricular work, expressed as the product of heart rate and systolic aortic pressure (33). It has also been shown that the effect of propranolol in angina pectoris is more pronounced in cases with a low working capacity and a high pulse rate in standing position (15), as can be found in untrained individuals. Group B had a higher PRP at rest and a higher heart rate for a certain increase in V_{O_2} than group C, a finding which would fit in with this hypothesis. Beta-adrenergic blockade is more effective in cases with hyperkinetic circulation (8).

A high LA pressure at work was found in all cases except case 11. This increase was slightly less in group B before alprenolol. The normal pressures in case 11 were not due to low output, since the minute volume was normal at rest and rose normally during exercise (Table II). This patient died in myocardial infarction 6 months later which leaves little doubt concerning the nature of his disease. A higher work load would probably have been tolerated and resulted in more pain and increased LA pressure. This might also apply to occasional cases in other studies, with pain but a normal left ventricular filling pressure (13, 26, 42).

The increase in left ventricular filling pressure is a sign of reversible myocardial failure secondary to acute coronary insufficiency. There is a correlation between the pressure rise at exercise and left ventricular dimensions at rest (5) indicating a contribution of irreversible myocardial fibrotic changes. The high pulmonary artery pressures during exercise suggest that right ventricular work is also of importance.

Since pronethalol and propranolol were introduced in clinical practice (17, 31) the hemodynamic effects have been studied also in cases with ischemic heart disease, at rest and during exercise (2, 13, 18, 29, 30, 32, 37, 42, 44). The injection of 5 mg propranolol resulted in a decrease in heart rate and cardiac output, while pulmonary artery pressure increased both at rest and during exercise, suggesting increased left ventricular filling pressure due to the negative inotropic effect (18, 37). When nitroglycerine was compared with 0.1 mg/kg propranolol i.v., a rise was found in left ventricular end-diastolic pressure at rest (32).

Repeated exercise tests were performed in 9 cases with angina pectoris, before and after 10 mg propranolol i.v. (13). Four out of nine cases, all having angina at the first exercise, could perform the same work without angina, but with the same rise in left ventricular end-diastolic pressure as before. The 5 cases who reacted with angina also after propranolol had the same rise of pressure. The negative inotropic and chronotropic effects were more marked in those without pain in the second test, a difference of interest with regard to similar comparisons in the present study. In a similar study with a larger dose of propranolol (0.15 mg/kg) all cases had angina during exercise before as well as after β-blockade (30). Cardiac index and heart rate decreased significantly after propranolol, both at rest and during work. The end-diastolic left ventricular pressure rose significantly at rest, but the increase during work was

unchanged. In another study on 8 patients the exercise-induced angina was abolished after propranolol 5–10 mg i.v. (2).

The effect of β-adrenergic blockade on heart size or left ventricular volume has been studied, but the results hitherto seem controversial (1, 36, 41, 43).

Clinically alprenolol has proved to be effective in the treatment of angina pectoris (4, 7, 35). When given intravenously propranolol and alprenolol are equipotential and also have the same time-effect relationships (21). The hemodynamic effect at rest of alprenolol compared with propranolol was studied on 5 young healthy men (14). Although 10 mg i.v. of both drugs was equipotential with regard to isoprenaline, alprenolol caused no significant changes in basal hemodynamics, while propranolol resulted in a significant fall in cardiac output. This difference was ascribed to the intrinsic β-adrenergic stimulant action of alprenolol (1, 9, 21).

In this study alprenolol was given to 12 cases, with a significantly lower rise of heart rate and PRP during exercise after alprenolol. Whether the changes noted in LA and pulmonary arterial pressures during exercise are less than those found with propranolol is difficult to assess because of differences in patient selection, drug dosage and methods of analysis. The group of 4 cases with less effort angina after alprenolol also had a slightly lower LA pressure rise in the second work period. This observation contrasts with findings in some earlier studies with propranolol (13, 30) but confirms others (2). Studies on the effect of repeated exercise tests on hemodynamics during exercise have shown a small decrease in pulmonary artery pressure in normal cases (11) and in cases with angina pectoris (26).

The importance of optimal dosage in the peroral treatment of angina pectoris with alprenolol has been demonstrated (35). A similar individual dose dependence is to be expected also for i.v. administration.

The clinical importance of the β-adrenergic stimulant action of alprenolol is not yet clear. It has been demonstrated that alprenolol compared with propranolol has considerably less effect on airway resistance (34, 40). In a patient with chronic bronchitis and angina pectoris alprenolol should be preferable, although it must be given with great caution in this situation.

Alprenolol had no negative inotropic effects in healthy men (14). Such an effect has, however, been demonstrated in patients with heart diseases (19, 23, 25), where the adequacy of circulation might depend on the sympathetic drive. For the same reason β-adrenergic blocking drugs may have a profound negative effect during anesthesia (39). As they act by means of competitive inhibition, isoprenaline should be given as an antidote. Glucagon has been reported to be effective (10).

The main therapeutic value of β-adrenergic blocking drugs for angina pectoris is in the long term peroral prophylactic medication. Compared with nitroglycerine they are less effective when short-term hemodynamic studies are concerned (3, 32). For such studies physical exercise is the most physiological test. However, during exercise it is sometimes difficult to obtain angina of sufficient severity to be significant and yet to be tolerable for the patient without undue discomfort during the period necessary for recording physiological events. It is possible that overdriving with right heart pacing (38) is preferable.

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Acta med. scand. Vol. 193 pp. 419–4.4 1973

ALPRENOLOL ALONE AND IN CONJUNCTION WITH PENTANITROL IN ANGINA PECTORIS

A Double-blind Study with Exercise Tests

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Abstract. Total work performance has been determined in 13 patients with angina pectoris during treatment with alprenolol alone and in conjunction with pentanitrol in double-blind study Nine patients had significant increase in work performance on alprenolol, but there was no difference between the two preparations. By measuring triple product and QS$_2$ index before and after test dose of alprenolol, it may be possible beforehand to select responders to this treatment.

The beneficial effect of several adrenergic β-blocking agents in angina pectoris is now well established (2, 5, 6, 7 8, 17 18). In most studies the frequency of anginal attacks and the nitroglycerin consumption ha e been used as main variables. However these parameters need not necessarily reflect the degree of severity of angina, as has been pointed out by others (9 14 15) The subjective symptoms are in general dif ficult to quantitate, and exercise tests to determine work tolerance ha e often been used (1 4 13) We chose to use repeated exercise as our main variable because we think this provides the best way of quantitating a therapeutic response in angina pectoris.

The study was also undertaken to evaluate older antianginal treatment which might ha e a mode of action different from β-blockers. Aubert et al. (3) showed that pentanitrol (PETN) in dose of 30 mg q.i.d. only occasionally improved angina patients, and that it was significantly less effective than alprenolol (ALP). We therefore chose to study this drug in conjunction with ALP to see whether the effect of such a combination could be significantly different from that of a β-blocker alone, as has been suggested by Russek (11)

MATERIAL

General practitioners were asked to send their patients ith effort angina to the study The recruitment has been slow and only about 30 patients ere admitted. A careful selection was made according to the following criteria. typical angina pectoris on effort, with uniform pattern during at least 6 months, reasonably high frequency of attacks, and responding to nitroglycerin. Patients with frank congestive heart failure and with nocturnal dyspnoea, AV block, valvular heart disease and diabetes ere not admitted to the study T enty four patients met these criteria and started the exercise tests, but of these 11 dropped out, so that only 13 patients completed the study Some anthropometric data are given in Table I. All patients had normal Hb concentration.

The reasons for exclusion of cases already in the study ere as follows: during the control period one patient de eloped congesti heart failure, two had no angina during exercise tests and one could not use the bicycle. During the single-blind placebo period two patients developed conduction disturbances and heart failure, one had an eye operation, hereas two ere excluded because of lack of cooperation (tablet failure). During the double-blind periods one patient stopped ALP on several occasions because of dyspnoea and fatigue. ALP had no significant effect on angina or performance in this case. Another patient experienced excessive fatigue and depressed mood during the second period of ALP she had no angina pectoris on this medication.

METHODS

The design of the trial was as follow: 2 weeks on nitroglycerin sublingually 4 weeks on single-blind placebo (placebo$_{sb}$) q.i.d. and nitroglycerin. Three periods of 4 eeks each with (double-blind): ALP 100 mg q.i.d. and

Table I. *Data on the 13 patients who completed the study*

Abbreviations. A = alprenolol, AP alprenolol + PETN, Pl = placebo

Case no.	Duration of angina (y)	Resting ECG	Heart size (cm^3/m^2)	Cholesterol (mg %)	Nitroglycerin control period (tablets/week)	Order of tablets
1	1.5	Neg. T_{V4-6}	490	331	15.0	AP A Pl
2[a]	3	LBBB	700	291	5.5	A AP Pl
3[d]	10	Post infarction	560	337	10.0	A Pl AP
4	0.5	Normal	480	319	8.5	Pl A AP
5[a]	1.25	Left axis	580	347	14.5	A AP Pl
6	10	Depressed ST_{V4-V6}	750	—	33.5	AP A Pl
7	1	Normal	530	—	2.0	Pl A AP
8[c]	1.2	Post infarction	630	269	11.5	AP Pl A
9	6	Depressed ST_{V4-V6}	430	324	3.5	Pl AP A
10[d]	4	RBBB	820	215	30.5	Pl A AP
11[b]	5	LV hypertrophy	490	272	19.0	A AP Pl
12[d]	7	Post infarction depressed ST_{V4-V6}	830	267	2.5	A AP Pl
13[a]	11	Normal	540	322	32.0	AP Pl A

Drugs: [a] digoxin, [b] aldomet thiazide. No. of myocardial infarctions: [c] 1, [d] 2.

nitroglycerin, ALP 100 mg + PETN 30 mg q.i.d. and nitroglycerin, double-blind placebo ($placebo_{AA}$) and nitroglycerin, in randomized order with double-blind allocation.

The patients were asked to use nitroglycerin if anginal attacks occurred, but never as prophylaxis. Placebo or ALP tablets were taken 90 min before the exercise test was performed. Tests were made every second week during the trial, two tests on every single drug preparation. The exercise test was performed on a bicycle ergometer with accurate electrical calibration of work load. Measurements were made with the patient sitting on the bicycle at rest, during and after exercise. The patients started with a work load of 150 or 300 kpm/min, and the load was increased in steps of 150 kpm/min when steady state was reached, determined by constant pulse rate ± 2 beats/min during 1 min. Three patients were familiar with the bicycle ergometer from an earlier study in which exercise tests were used once every third month.

ECG was monitored during and after exercise, using three CR leads which allow stable ECGs to be taken during exercise. A phonocardiogram was taken with the microphone placed in the praecordium where the second aortic sound was loudest. BP was measured by the cuff method before and immediately after the end of exercise.

A clinical examination was made at every test and the patients were asked about the subjective effect of the drug and possible side-effects.

The exercise test was stopped when the patient experienced angina of moderate to severe intensity and he was asked to reach the same intensity of pain every time. Angina pectoris was the reason for stopping exercise in all except 6 instances, when exercise was stopped owing to exhaustion. Depression of ST segments in the ECG was never used as an indication for stopping exercise. Some of the patients received nitroglycerin immediately after exercise to relieve severe angina.

The patients recorded daily on special diary cards the attack rate and the nitroglycerin consumption during the trial.

Statistical methods

Three-dimensional analysis of variance was performed on all variables listed in Table II, except those concerned with the ECG. There was no significant occasion variance for any parameter. The following combination variances were significant: occasions, patients and treatments; patients for heart rate at end of exercise ($p < 0.05$), occasions, drugs for diastolic BP at end of work ($p < 0.05$) and patients, drugs for nitroglycerin consumption per week ($p < 0.001$). With Scheffé's contrast, individual confidence limits were determined. In the cases where sufficient values from the $placebo_{AA}$ period were available for analysis, it was seen that the means from this period were never significantly different from the means during the $placebo_{AA}$ period. Furthermore, the ALP period means never differed significantly from (ALP + PETN) period means.

ECG changes were analysed by the Wilcoxon matched pairs signed-ranks test, two-tailed, as test for normality revealed non-normal distribution.

RESULTS

Angina pectoris was produced during all exercise tests on placebo, during all tests on ALP except one patient (no. 9), whereas three patients (nos. 5 and 9 (both tests) and no. 6 (one test)) ex-

Table II. *Results of the statistical analyses*

Values for ALP are never significantly different from values for ALP+PETN. Patients 2 and 3 are not included when $N<13$. For ECG normalization time also patient 4 is excluded due to lack of data

	Placebo$_D$	ALP	ALP+PETN	Confidence limit
Total work (kpm)	1 251	1 987	2 022	$p<0.001$
Heart rate				
At rest	73.7	64.5	64.6	$p<0.001$
At end of work	119.3	105.4	106.2	$p<0.001$
Systolic BP				
At rest	139.6	141.3	140.4	N.S.
At end of work	159.2	156.2	153.1	N.S.
Diff. work–rest	*21.2*	*14.8*	*12.7*	$p<0.05$
Diastolic BP				
At rest	84.4	83.7	82.5	N.S.
At end of work	88.2	84.0	82.7	$p<0.05$
QS$_2$ index at rest	0.55	0.55	0.54	N.S.
Triple product at rest	403	373	377	N.S.
Triple product at end of work	593	559	561	N.S.
ST depression at beginning of angina (mm)	0.20	0.15	0.16	$p<0.05^a$ $N=11$
ST depression at end of work (mm)	0.22	0.18	0.20	$p<0.05^a$ $N=11$
Time to 1 mm ST depression (sec)	114	156	159	N.S. $N=11$
ECG normalization time (min)	4.3	4.0	5.0	N.S. $N=10$
Duration of pain after work (sec)	*143*	*146*	*105*	N.S. $N=12$
Nitro/week	11.5	6.3	5.2	$p<0.05$

[a] Wilcoxon matched pairs signed-ranks test, two-tailed.

perienced no pain during exercise on ALP+PETN.

Total work during control periods and periods with placebo$_{SB}$ and placebo$_{DB}$ showed no significant difference. There seemed to be a larger difference between the first control test and the five other tests during control and placebo periods (mean $\pm$S.E.M. for first test 1 058 $\pm$ 135.9 kpm and for five tests 1 281 $\pm$ 90.1 kpm), but the difference was not significant.

With regard to nitroglycerin consumption there

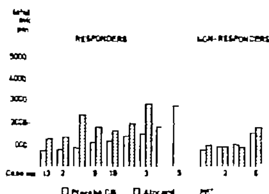


Fig. 1. Total work during placebo$_{DB}$ and ALP periods.

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There was no significant difference between the first and the second test in any of the periods.

As a whole there was no significant difference tested by variance analysis between findings during periods with ALP alone and periods with ALP+PETN. Therefore the results from these two periods are taken together versus placebo$_{DB}$. The results of the statistical analyses are shown in Table II.

Total work

This was calculated as the product of kpm/min and number of minutes of exercise. The mean increase in total work on ALP medication was 58.2%. In seven patients the increase in total work was more than 50%, in two patients 25–50%, whereas it was only slightly increased or *decreased in four patients (−11%–+16%).* These four patients were regarded as non-responders (Fig. 1 and Table III). There was no correlation between total work performance and heart size or cholesterol (Table I).

Heart rate

Heart rate (HR) was measured from the ECG with the patient sitting on the bicycle. At rest the HR decreased from 74 min to 65 min on ALP

Table I. *Data on the 13 patients who completed the study*

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Drugs: digoxin, aldomet thiazide. No. of myocardial infarctions 1, [d] 2.

nitroglycerin, ALP 100 mg PETN 30 mg q.i.d. and nitroglycerin, double-blind placebo (placebo$_{pa}$) and nitroglycerin, in randomized order with double-blind allocation.

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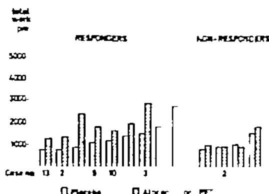


Fig. 1. Total work during placebo$_{DB}$ and ALP periods.

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As a whole there was no significant difference tested by variance analysis between findings during periods with ALP alone and periods with ALP+PETN. Therefore the results from these two periods are taken together versus placebo$_{DB}$. The results of the statistical analyses are shown in Table II.

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This was calculated as the product of kpm/min and number of minutes of exercise. The mean increase in total work on ALP medication was 58.2%. In seven patients the increase in total work was more than 50%, in two patients 25–50%, whereas it was only slightly increased or decreased in four patients (−11%–+16%). These four patients were regarded as non-responders (Fig. 1 and Table III). There was no correlation between total work performance and heart size or cholesterol (Table I).

Heart rate

Heart rate (HR) was measured from the ECG with the patient sitting on the bicycle. At rest the HR decreased from 74/min to 65/min on A-

Table III *Effect of medication on total work*
Abbreviations as in Table I

Case no.	Subjects effect	Increase in total work (%)	Triple product at rest PI$_{DB}$	A AP	Difference A PI (%)	QS$_2$ index at rest PI$_{DB}$	A AP	Difference A–PI
1	Excellent	+104	4 600	3 900	−15	0.56	0.57	+0.01
2	None	86	3 700	3 200	−14	0.55	0.55	0.00
3	Excellent	+ 91	3 700	3 350	− 9	0.56	0.56	0.00
4	Excellent	− 35	4 000	3 750	− 6	0.58	0.55	−0.03
5	Excellent	− 73	4 100	3 900	− 5	0.54	0.53	−0.01
6	Excellent	− 16	3 850	3 850	0	0.51	0.53	+0.02
7	None	− 11	3 850	4 450	+16	0.54	0.57	+0.03
8	None	− 15	3 550	3 650	+ 3	0.54	0.54	0.00
9	None	− 70	3 250	2 600	−20	0.54	0.55	+0.01
10	None	+ 37	4 600	3 500	−24	0.55	0.52	−0.03
11	Excellent	+169	5 050	5 250	− 4	0.56	0.55	−0.01
12	Excellent	− 3	4 400	4 700	+ 7	0.56	0.58	+0.02
13	Good	+ 70	4 050	3 650	−10	0.56	0.54	−0.02

The maximal HR at end of exercise also decreased on ALP from 120 to 106/min. Angina pectoris started at a lower HR on ALP 106/min, than on placebo, 118/min. The differences are highly significant.

Blood pressure

Both systolic and diastolic BP were unchanged at rest. During exercise there was a significantly lower diastolic BP on ALP and the rise in systolic BP during exercise was much less.

QS$_2$ time (electromechanical systole)

QS$_2$ time was measured as the interval from the beginning of the Q wave in the ECG to the first part of the second aortic sound in the phonocardiogram. As QS$_2$ time is related to HR, a QS$_2$ index was calculated as QS$_2$ index = QS$_2$ + 0.0021 × HR (10, 16). The QS$_2$ index at rest decreased in 5 cases, increased or was unchanged in 8 cases on ALP (Table III).

Triple product

Systolic BP × HR × QS$_2$ time (called triple product, TP) is correlated to tension-time index and to myocardial oxygen consumption (12). A distinct decrease of 10% or more in TP at rest was seen in 5 patients on ALP. 3 showed a smaller decrease, whereas TP was unchanged or increased in 5 patients (nos. 6, 7, 8, 11 and 12) (Table III). During exercise almost the same pattern was demonstrated.

ECG changes

Only 3 patients had ST segment depression at rest. The duration of exercise to produce 0.1 mV ST segment depression, the magnitude of ST segment depression at angina and at end of work, and the ECG normalization time after exercise were measured. The ST segment depression at angina and at end of work was less pronounced on ALP medication than on placebo ($p < 0.05$) whereas the other parameters were not significantly altered.

Nitroglycerin consumption and subjective improvement

Nitroglycerin consumption decreased significantly during periods with ALP and ALP + PETN from 11.5 tablets/week on placebo to 5.8 tablets/week ($p < 0.05$). Four patients needed no nitroglycerin on ALP or ALP + PETN.

The overall subjective effect estimated by the patients was described as excellent by 7, moderate by 1, whereas 5 patients found no help from the drugs during everyday life. Most patients had the same estimate of ALP and ALP + PETN but 1 patient (no. 12) found ALP + PETN more satisfactory than ALP alone.

Side-effects and complications

As mentioned earlier, two patients were excluded during the trial due to signs of cardiac incompensation and fatigue. Furthermore, in patients 8 and 10, who both concluded the trial, minor

signs of cardiac incompensation were noted. Patient 8, a 74-year-old man, had slight cardiac incompensation during the last week of the trial, when he received ALP. As there was no subjective or objective effect of ALP the drug was stopped after the conclusion of the trial and anticongestive treatment was instituted. Patient 10, a 63-year-old man with enlarged heart, experienced dyspnoea during the second period of ALP and basal râle was heard. Treatment with digoxin was started, the symptoms disappeared and he continued in the trial. Insomnia was a disturbing side-effect in two patients, but disappeared when the tablets were taken at an earlier hour.

DISCUSSION

Treatment with ALP increased the physical work performance of 9 out of 13 patients. Addition of a long-acting nitrate compound PETN to ALP produced no significant difference in the objective findings, whereas one patient found the combination subjectively more satisfactory. This is in discordance with the findings of Russek (11) who found a definite effect, and of Adolfsson et al. (1) who found a moderate synergism between a β-blocking agent and isosorbide nitrate.

A placebo effect could be demonstrated in our material regarding the nitroglycerin consumption, which decreased during the placebo$_{\mathrm{AB}}$ period,

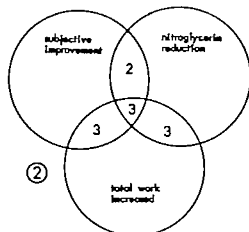


Fig. 2. Venn diagram illustrating cases with subjective improvement, nitroglycerin reduction and increase in total work during treatment with ALP.

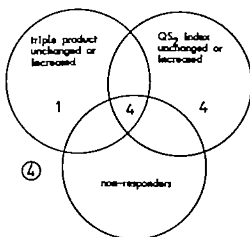


Fig. 3. Venn diagram illustrating cases where triple product and QS$_2$ index decreased at rest during ALP and cases without increase in total work during ALP.

but there was no difference in total work performance during the same periods.

On the basis of the increase in total work performance on ALP our material could be divided into two groups: 9 patients who increased their total work more than 25% (35–169%) and 4 patients who had a decrease or slight increase in total work (−11 to +16%) (nos. 6, 7, 8 and 12).

The four non-responders showed either no change or an increase in triple product at rest on ALP. In patients 6, 7 and 8 the decrease in heart rate on ALP was slight, in patient 12 the decrease was more pronounced. The QS$_2$ index increased in three of these cases and was unchanged in one. Increase in triple product points to an increased myocardial oxygen consumption, and a rise in QS$_2$ most probably means a lowered contractile state of the myocardium (10).

Eight of nine patients with increased work performance showed a decrease in triple product at rest on ALP, possibly due to a decrease of cardiac work load and of myocardial oxygen consumption at rest.

The same trends were seen in triple product at the end of exercise in the two patient groups, but it is doubtful whether the comparison is valid, as the physical work loads differed during placebo and ALP periods.

There was not complete concordance between the subjective and the objective effect as shown in Fig. 2. Thus, patients 2, 9 and 10 felt no overall

subjects a effect despite the fact that nitroglycerin consumption was significantly decreased by 32, 44 and 99% respectively and the physical performance was increased during exercise tests. The negative estimation of the treatment in these cases is most probably connected with the fact that two patients had slight cardiac incompensation (nos. 2 and 10) and patient 9 suffered from intermittent claudication during his daily work.

Two patients with no increase in work performance at exercise tests had an excellent subjective effect, as their attacks of angina pectoris were almost completely relieved on ALP during everyday activity.

These findings show that a primary selection of patients in whom a beneficial effect can be foreseen is very difficult. Some patients will have fewer angina attacks even if their work performance is unaltered, and patients on the verge of incompensation will feel no subjective effect despite an increase in work performance on ALP.

Fig. 3 shows the distribution of the patients according to the changes in triple product and QS_2 index at rest during treatment with ALP. It is seen that the four non-responders are characterized by an increase (or no change) both in triple product and in QS_2 index. Thus there may be a possibility to select patients who will respond to treatment with ALP by measuring these parameters at rest before and after a test dose of the drug.

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Acta med. scand. Vol. 193 pp. 425–429 1973

ATRIAL FIBRILLATION FOLLOWING THORACOTOMY FOR NON-CARDIAC DISEASES, IN PARTICULAR CANCER OF THE LUNG

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Abstract. A series of 300 consecutive thoracotomies for non-cardiac diseases has been analysed with a view to the occurrence and nature of postoperative cardiac arrhythmias. Atrial fibrillation proved to be by far the most common type. Among 163 patients who had undergone operation for malignant pulmonary disease 19.6% had atrial fibrillation postoperatively as compared with 3.1% of patients who had been operated upon for benign pulmonary disease ($p < 0.001$). More detailed assessment of the preoperative, operative and postoperative parameters in the group with cancer of the lung did not demonstrate factors predisposing to or eliciting atrial fibrillation. It is suggested that common aetiological factors—e.g. smoking—may play a role. Possibly investigations using constant monitoring of circulatory parameters may elucidate the pathogenesis of atrial fibrillation following thoracotomy.

Cardiac arrhythmia, in particular atrial fibrillation, is a very common complication following pulmonary surgery. This was pointed out first by Bailey and Betts in 1943 (1). Since that time this complication has been well known, but owing to the marked differences in the materials the incidence has ranged from 2% (10) to 21% (7), being even as high as 32.5% after pneumonectomy (5). Several surgeons have tried to avoid the arrhythmias by prophylactic medication. Hurt and Bates (8) have reported a definite effect of quinidine and Bergh et al. (2) obtained a significant prophylactic effect of digitalis following pneumonectomy.

In our department we have not used prophylactic medication against postoperative cardiac arrhythmias. The object of the present study was in part to ascertain the incidence of such arrhythmias and in part to investigate whether any groups of patients were particularly predisposed to atrial fibrillation during the postoperative period. Since atrial fibrillation was found to occur far most often in patients who had undergone operation for cancer of the lung, this group was used for analysing the influence of the parameters routinely recorded before, during and after the operation upon the occurrence of this arrhythmia.

MATERIAL

The material comprises 300 consecutive thoracotomies for non-cardiac diseases on 295 patients. During the same stay in hospital 5 patients had thoracotomy twice at several days' interval, the second operation being counted as a new thoracotomy. Two patients who were re-operated upon within 24 hours are counted only once. One patient who underwent bilateral thoracotomy under the same anaesthesia is counted as one case. We did not include cardiac arrhythmias arising during the operation. The day of the operation is taken to be zero day. No arrhythmias were demonstrated on this day. The analysis of arrhythmias is based exclusively upon the ECG findings. In the assessment we have used the definitions set up by the Criteria Committee of the New York Heart Association (6). However, sinus tachycardia was included only if the rate exceeded 140/min, and extrasystoles were not recorded as arrhythmias unless they occurred in bursts or coupled as bigeminy or trigeminy.

Preoperative investigations comprised ECG, chest X-ray, Hb, ESR, serum creatinine, pCO_2, oxygen saturation and base excess in arterial blood, and in most cases serum electrolytes. In the majority of patients who did not have an emergency operation, determinations of the lung volume, ventilation and helium mixing time were carried out. Postoperatively ECG and arterial blood analyses were performed on the 1st and 3rd day. Serum electrolyte studies were done with varying frequency depending upon the postoperative course. ECG was traced when the pulse count showed abnormalities, the pulse rate

Table I. *Incidence of atrial fibrillation in the various age groups following operation for cancer of the lung or benign pulmonary diseases*

Age (y.)		Cancer of the lung		Benign pulmonary diseases
0–49		2/14		0/29
50–59		10/46		1/16
60–69		17/78		0/12
[illegible]70		4/30		1/7
Total		33/168		2/64
	19.6	S.D. 3.1	3.1	S.D. [illegible]

Table II. *Number of patients with postoperative atrial fibrillation in relation to the total number at different FEV values among 162 patients with cancer of the lung*

	FEV (ml)				
	<1 000	1 000–1 499	1 500–1 999	2 000–2 999	>3 000
Atrial fibrillation/total	0/3	4/37	8/40	15/70	4/12

being checked at 15-min intervals on the day of the operation and on the 1st postoperative day thereafter at increasing intervals down to 2 daily counts. Chest X-ray done as routine on the 1st and 8th postoperative day and at other times in the event of complications.

RESULTS

Postoperative cardiac arrhythmias occurred in 62 patients or [illegible] of the entire series. Fourteen had sinus tachycardia, 4 had partial AV block, in 5 patients extrasystole was present and one patient showed nodal rhythm. Atrial fibrillation was by far the most common type being found in 38 patients (1[illegible]7 %). This type of arrhythmia was subjected to a more detailed analysis.

If the material is classified by organ affected and by the criterion benign malignant disease we found that 33 of 168 patients with lung cancer had atrial fibrillation against 2 of 64 patients with benignant pulmonary disease. The difference in the incidence of postoperative atrial fibrillation in patients with malignant (19.6 %) and with benign (3.1 %) diseases of the lung was significant ($\chi^2 = 9.87$, $f = 1$, $0.005 > p > 0.001$). Among patients who underwent thoracotomy for diseases of the oesophagus or diaphragm the incidence of atrial fibrillation was low. Only one out of 18 patients with oesophageal cancer and 2 out of 50 with benign diseases in the oesophagus and at the diaphragm got atrial fibrillation.

The incidence of atrial fibrillation following pneumonectomy, lobectomy and minor procedures including exploratory thoracotomy was not very different. 1[illegible] of 54 ([illegible] %), 3 of 64 (20.3 %) and 8 of 50 (16.0 %) respectively got atrial fibrillation.

The extent of the operation was greater in patients with pulmonary malignancy. This is not taken into account by a direct comparison of the two groups. However, a comparison of the incidence of arrhythmia in patients having thoracotomy for benign pulmonary diseases (2 out of 64) with that in patients having exploratory thoracotomy or minor resection for cancer of the lung (8 of 50) still shows a significant difference ($\chi = 5.81$, $f = 1$, $p < 0.05$).

As is apparent from Table I, in the age group under 50 there was a considerable preponderance of patients who had undergone operation for benign pulmonary diseases. This may effect the incidence of postoperative atrial fibrillation. Analysis of the over 50 group showed a significantly higher incidence of atrial fibrillation, also

Table III. *Incidence of atrial fibrillation following operation for cancer of the lung with increasing duration of the operation*

	Duration of operation (h)			
	<1	1–2	2–3	>3
atrial fibrillation/total	0/6	9/51	15/72	9/39

Table IV. *Incidence of postoperative atrial fibrillation at different magnitudes of operative blood loss*

	Blood loss (ml)				
	<250	250–499	500–999	1 000–1 999	>2 000
+ atrial fibrillation/total	4/30	12/48	11/58	5/23	1/6

when considering only the group with cancer of the lung ($\chi^2 = 4.11$ $f = 1$ $p < 0.05$). The mean age of patients with benign diseases of the lung was 61.5 years (range 50–74) and that of patients with cancer of the lung 63.5 years (range 50–78). There was no difference in the incidence of atrial fibrillation among the 28 women and 140 men who underwent operation for cancer of the lung, as it occurred in 4 of the women and 29 of the men. The unequal sex ratio is normal for series of lung cancer

Preoperative findings

In patients with a history of previous admission or treatment for cardiac disease, cardiac symptoms such as anginal pain, signs of congestive heart failure or exertional dyspnoea prior to the present pulmonary disease 10 out of 36 had atrial fibrillation. In 3 patients with hypertension (BP > 170/100) 20 patients with X-ray signs of cardiac ectasia (index > 0.5) and 65 patients with ECG abnormalities prior to operation, 1 2 and 12 patients, respectively got an atrial fibrillation. Among the patients with a preoperatively abnormal ECG 31 had isoelectric or negative T I, 6 of whom (19.4%) developed atrial fibrillation. 137 patients had a normal T I of whom 27 (19.7%) developed atrial fibrillation. Among 72 patients, whose cardiac status had been normal prior to the operation, 13 developed atrial fibrillation, as compared with 20 among 96 patients who had exhibited one or more of the named abnormalities before the operation.

FEV_1 being one of the important parameters of pulmonary function, is related to atrial fibrillation in Table II. There is no correlation between FEV and the incidence of arrhythmia. No lung function tests had been done in 6 cases, 2 of whom developed atrial fibrillation.

Findings during operation

Minor operations are taken to be segmental and subsegmental resections and exploratory thoracotomies for taking a biopsy

Eighty-seven left-sided and 80 right-sided thoracotomies were performed. Within the former group there were 13 instances of atrial fibrillation and within the latter group 20. In other words, there is a slightly increased incidence of arrhythmia among the right-sided cases, but the difference is not significant ($\chi^2 = 2.66$, $f = 1$ $0.1 < p < 0.2$). A patient who had bilateral thoracotomy in the same stage is not included.

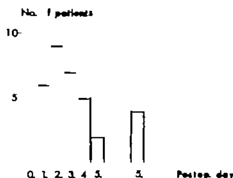


Fig. 1 Time of onset of postoperative atrial fibrillation in 33 patients operated upon for cancer of the lung.

In 64 patients there was pericardial involvement. Fifteen of them were inoperable because of invasion by cancer and the pericardium was not opened. In all the others the pericardium was opened, i.e. in cases of exploration resection of the pericardium, or when vessels were sutured close to the atrium. Twelve of the 64 patients with pericardial involvement developed atrial fibrillation, whereas only 21 of 104 patients without pericardial involvement developed this postoperative complication.

The duration of the operation and the magnitude of the blood loss did not show any findings that could be correlated to the occurrence of atrial fibrillation (Tables III and IV) In 3 cases the blood loss was not measured.

We did not analyse the influence, if any of the anaesthetics upon the incidence of atrial fibrillation, as any effect of the various anaesthetics must be presumed to have been eliminated at the time when the arrhythmias arise As is apparent from Fig. 1 no patient developed arrhythmia on the day of the operation, and the incidence was highest on the 2nd and 3rd day

Postoperative findings

One out of 17 patients with elevated pCO_2 (exceeding 43 mm) developed atrial fibrillation as compared with 32 out of 151 patients with normal pCO_2. The oxygen saturation of the arterial blood was below 90% in 48 patients, 7 of whom developed atrial fibrillation. Out of 114 patients with normal oxygen saturation 26 had atrial fibrillation. Base excess was normal in 62 patients,

16 of whom developed atrial fibrillation. Seventy eight patients were acidotic (base excess < – 2.3) and among them 13 developed atrial fibrillation. Among 26 alkalotic patients (base excess > + 2.3) 4 developed atrial fibrillation. Hb level below 80% was found in 11 patients, 3 of whom developed atrial fibrillation. The remaining 157 patients had Hb levels exceeding 80 and 30 exhibited atrial fibrillation. There was no definite relationship between the occurrence of atrial fibrillation and disturbances of serum potassium. One hundred patients had a transient elevation of temperature up to 38–39 C during the postoperative period. Only 9 developed atrial fibrillation, while the temperature was within this range. In 70 patients the temperature was 37–38 C at the onset of the arrhythmia.

Course of arrhythmias

Fig. 1 shows the time at which the postoperative atrial fibrillation arose. It will be seen that 27 out of 33 patients developed their atrial fibrillation within the first 4 postoperative days, as a rule with rapid ventricular rate. In 26 of the 33 patients the ventricular rate exceeded 150/min at the onset of the arrhythmia. In many cases the arrhythmia was short-lasting, in 12 cases less than 4 hours. In 16 patients the arrhythmia persisted for longer than 3 days, and within this there were 9 who had more than one ode

Treatment

As a rule the arrhythmia was treated by quick i digitalization (6 patients). However oral digitalis medication was started in 3 patients and one received a β-receptor blocking agent (Aptin®) during the acute phase. Three patients did not have any treatment. One was already on digitalis, and in the other the arrhythmia was brief. In 5 cases quinidine was used later in the course, and in 4 β-receptor blocking agents. At the time of discharge a total of 70 patients were on oral digitalis, 4 on quinidine and 3 on β-receptor blocking medication. All the patients with postoperative atrial fibrillation had reverted to sinus rhythm when discharged.

Postoperative complications

Among the 168 patients operated on for cancer of the lung there were 8 deaths during the stay in hospital (4.8%). There was one death among the patients with atrial fibrillation, so that in this group there was no excess mortality. This patient died 3 weeks after a palliative operation in a poor general condition with metastases from the lung cancer.

As regards serious, but non-fatal postoperative complications in the group with atrial fibrillation, there was 1 case of coronary occlusion, which occurred in 3 of the remaining patients with cancer of the lung during the postoperative period.

DISCUSSION

The present investigation revealed that atrial fibrillation was the most common type of arrhythmia following thoracotomy. Sinus tachycardia was also fairly common but during the postoperative period it may be due to factors such as pain, hypovolaemia or fever and need not always represent a cardiac affection. As pulse counts were made at such brief intervals, we believe that we have detected most cases of atrial fibrillation, whereas the demonstration of extrasystoles may have been incomplete, as continuous monitoring was not employed. For these reasons we had to restrict the more detailed analysis to comprise only the findings relating to the postoperative atrial fibrillation.

In this analysis our first finding was a significantly increased occurrence of atrial fibrillation following thoracotomy for cancer of the lung as compared with thoracotomy for benign pulmonary diseases. This has been reported previously without being attributed any major importance (3, 7, 12). However it has been related to a simultaneous higher frequency of operations involving the pericardium (2). In our opinion it has not yet been elucidated whether it is the cancer of the lung in itself or its aetiology—e.g. smoking—which predisposes to an increased incidence of atrial fibrillation, whether special factors relating to the operations elicit the arrhythmia with increased frequency or whether both factors are operative.

It is difficult to compare patients operated upon for benign and for malignant pulmonary diseases, as the type of operation differs. Among other things, pneumonectomy is rarely performed for a benign disease. Therefore the materials must be corrected for differences in the type of

operation. By comparing all patients who underwent operation for a benign pulmonary disease with patients who had exploratory or minor operation for lung cancer we have placed the more extensive operations in the benign group, but nevertheless the incidence of atrial fibrillation is higher among the patients with cancer of the lung. We also compared the two groups after paying regard to age, excluding patients under 50. This, too, did not alter the fact that atrial fibrillation is more common among patients with cancer of the lung.

Having a material of 168 patients with cancer of the lung, about 20% of whom developed atrial fibrillation, we ought to be able to demonstrate predisposing or eliciting factors.

In some materials (1 4 5 12) the incidence of atrial fibrillation is higher following pneumonectomy than following lobectomy but this could not be confirmed in our study However there may be differences in the materials if pneumonectomy has been used as the standard operation and if preserving methods, such as lobectomy with bronchial resection, have not been employed (15). Pericardial involvement, in the form of tumour invasion or opening of the pericardium, possibly suturing vessels close to the atrium, would be expected to make for a more common occurrence of atrial fibrillation because of vagus irritation and aseptic pericarditis (2, 8, 11 14) These factors we were also unable to confirm. *Nor could* we demonstrate a relationship between the incidence of atrial fibrillation and the duration of *the operation or the magnitude of the operative* blood loss.

It was not possible, either in other respects to demonstrate predisposing or eliciting factors. In particular we did not find an increased incidence of atrial fibrillation in patients with a history of heart disease prior to the operation.

In the further study of the arrhythmias more intensive investigations must be carried out, similar to those made by Johansson et al. (9) but with constant monitoring of haemodynamic parameters in order thereby to elucidate the period immediately preceding the onset of the arrhythmias

By means of postexercise ECG the diagnosis of preoperative heart disease may be improved. Thereby it is perhaps possible to demonstrate a correlation between preoperative cardiac disease and the risk of atrial fibrillation during the post operative period.

We do not advise prophylactic treatment with quinidine or digitalis (2, 8, 13). Using digitalization immediately after the onset of atrial fibrillation we have not had an increased mortality among the group of patients exhibiting this type of arrhythmia.

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Acta med. scand. Vol. 193 pp. 431–436, 1973

THROMBOCYTOSIS

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Abstract. The numbers of circulating platelets have been determined in series comprising 4 003 hospitalized patients. An elevated platelet count (>400 000/μl) was found in 222 cases. *Frequency figures of thrombocytosis in* excess of 30% found in myeloproliferative disorders, amyloidosis, ulcerative colitis, rheumatoid arthritis, ankylosing spondylitis and Hodgkin disease. In several diseases the cause of an increased number of circulating platelets is obscure, and the term "reactive thrombocytosis" is often applied. It is noteworthy that high frequency of elevated platelet counts was found in diseases in which immunological mechanisms are known or assumed to play part in the pathogenesis. The results obtained are briefly discussed in relation to those from earlier investigations, and an indication is given of the diagnostic implications of an increase in platelet value.

The number of circulating platelets in healthy individuals varies within a rather narrow range. Of the conditions characterized by altered platelet level, the thrombocytopenias are well known. Although the occurrence and the significance of elevated platelet counts are not understood to the same degree, marked thrombocytosis is often followed by thrombosis and/or haemorrhagic manifestations.

Normally about one-third of the platelet mass is concentrated in the spleen (1). A change in equilibrium between the splenic pool and the re*maining two-thirds of the platelets in the vascular* system may lead to thrombocytosis, which is particularly observable after splenectomy. An increased production of platelets also leads to thrombocytosis. Theoretically thrombocytosis may develop as a consequence of a prolonged life-time of platelets, but no well-documented cases provide evidence that this mechanism exists (6).

The purpose of this investigation has been to study the diseases in which elevated platelet counts are found, and the extent of such changes, among the patients in a department of internal medicine of a university hospital.

MATERIAL

During the period 1969–1970, 4 333 patients were admitted to the department. Patients receiving corticosteroids or immuno-suppressive drugs have been excluded, as have patients with signs of haemoconcentration arising from dehydration *(330 patients). The final series consists of* 4 003 patients.

METHOD

The platelets in blood, obtained by venous puncture, were counted as routine measure by application of the direct method described by Björkman (3). The original report gave the mean value of thrombocytes in healthy persons as 259 240 ± 5 200/μl within the range of 155 000–345 000. These values are in conformity with those found in our laboratory: mean value 292 000 ± 6 100 (S.E.), S.D. 46 000 ($n = 60$), range 180 000–385 000. In this study platelet counts in excess of 400 000/μl were considered to be elevated.

RESULTS

An elevated platelet count was found in 222 patients. The distribution by disease has been listed *in Table I. About one half of the* patients with an increased platelet value had thrombocytes between 400 000 and 500 000/μl. Twenty-two patients had values exceeding 700 000, of whom 10 were suffering from a myeloproliferative disease. The highest value noted was 2 200 000 in a case of polycythaemia vera. Values in excess of 1 000 000 were found in 4 other cases (1 case of chronic granulocytic leukaemia, 2 cases of polycythaemia vera, and 1 case of primary thrombocythaemia).

patients had an active disease that necessitated hospitalization. No clear relationship was demonstrable between platelet counts and activity. In most cases intestinal bleeding occurred, but no correlation existed between haemoglobin values and platelet counts. Consequently posthaemorrhagic anaemia can be excluded as a cause of the thrombocytosis.

Thrombocytosis was also found in 2 patients of 5 with Crohn's disease and in 3 other cases of chronic colitis, of the last-mentioned patients . had protein-losing enteropathy

Insufficient knowledge is possessed of the cause of thrombocytosis in chronic bowel diseases. However strong evidence exists that immunological features play a part at least in ulcerative colitis, and possibly in Crohn's disease as well. It may be that thrombocytosis belongs to these immunological alterations.

Liver diseases

Elevated platelet values have been noted in chronic alcohol cirrhosis of the liver (14 21), although thrombocytopenia is of more frequent appearance. Aetiological speculations have included coexistent sideropenia, normalization of the dietary folic acid content during hospitalization, and a rebound effect after thrombocytopenia when the alcohol intake has been stopped. No correlation has been found between the platelet count and the severity of liver damage, as determined by liver function tests, and the histological examination of biopsy specimens (14).

In the present series thrombocytosis was found at a somewhat higher frequency in chronic liver disease (16) than in acute hepatitis (10)

Since thrombocytosis is observable in different types of liver disease it seems evident that none of the alterations mentioned above provides a full explanation of the underlying causative mechanism. The occurrence of thrombocytosis is not particularly frequent and its cause may differ appreciably in individual patients. However it is worthy of note that in this series 4 of the 7 patients with chronic liver damage had chronic aggressive hepatitis, in which immunological phenomena are demonstrable

Bleeding and sideropenia

It has been well documented that acute bleeding is followed by initial thrombocytopenia which does not correspond to the severity of the bleeding, and 1 to 2 weeks later by secondary thrombocytosis (4). Moreover elevated platelet values have been recognizable in sideropenic anaemia without bleeding (.3). In these cases the cause of the thrombocytosis seems to be the mobilization of a megakaryocyte stimulating plasma factor which also induces similar thrombocytosis in other thrombocytopenic patients (19).

In this series thrombocytosis was found in 19 patients with recent haemorrhagia. On the other hand thrombocytosis was not noted in 8 patients with sideropenia but no other underlying disease. It thus seems that thrombocytosis appears more frequently in posthaemorrhagic anaemia than it does in pure iron-deficiency anaemia.

Rheumatoid arthritis and ankylosing spondylitis

Reports have been published on elevated platelet values in a few patients with rheumatoid arthritis (2, 10 12, 14 21). In the present series thrombocytosis was found in 29% of patients with this disease. In a previous investigation it was found that the thrombocytosis was correlated with the activity of the disease the presence of rheumatoid factor anaemia, sideropenia and leukocytosis (20). In rheumatoid arthritis with secondary amyloidosis (15 patients) an even higher frequency of thrombocytosis was observable (53%). Nevertheless no correlation was observable between the presence of renal insufficiency and/or proteinuria with increased platelet values. The thrombocytosis in rheumatoid arthritis seems to be attributable to immunological activity and not to anaemia or sideropenia of simultaneous occurrence (20).

In patients with ankylosing spondylitis thrombocytosis was found in the same frequency as in rheumatoid arthritis (32). In these patients no anaemia, sideropenia or rheumatoid factor was demonstrable, which also indicates that these factors are negligible in the pathogenesis of thrombocytosis in both ankylosing spondylitis and in rheumatoid arthritis.

Diseases of the connective tissue (excluding rheumatoid arthritis)

This group includes patients with systemic lupus erythematosus, periarteritis nodosa, scleroderma, dermatomyositis and Sjogren's syndrome. Of 6. patients who had not been given corticosteroid.

or immunosuppressive drugs, 9 were found to ha e elevated platelet alues, these 9 patients did not differ from the others in respect of haemoglobin values or the severity of the disease, although all of them had marked joint symptoms. The cause of the thrombocytosis in these cases is not understood, but it is possible that co-existent rheumatoid inflammation was in progress at the time of in estigation. In general, thrombocytosis is a rarer finding in these types of connective tissue diseases than are increased platelet values in rheumatoid arthritis.

Infections

Elevated platelet values have been observable in a number of acute and chronic infections (14 17). Thrombocytosis in tuberculosis patients is not rare (5 21). In acute viral infections thrombocytopenia is often found during the initial phase by reason of the aggregation of platelets at circulating iral antigen-antibody complexes, followed by reactive thrombocytosis.

In the series studied, thrombocytosis was observed in 17 cases in which the infection was the main disease: bacterial pneumonia in 8 cases, tuberculosis in 2, chronic bronchitis with acute exacerbation in 2, severe wound infection in 2, and tonsillitis, cholecystitis and osteomyelitis in 1 case each. When it is borne in mind that 172 patients were examined in this group the frequency of thrombocytosis was not high.

Renal diseases

A diminished number of platelets is a common finding in chronic renal diseases with impaired renal function. However thrombocytosis has also been observable without any relationship being apparent between platelet levels and the degree of renal insufficiency (14).

In the present series 314 patients with chronic nephritis were examined, one half of them had impaired renal function. In only a few cases were elevated platelet alues discernible in both groups. Patients with acute nephritis (interstitial nephritis and glomerulonephritis, with and without coexistent nephrotic syndrome) exhibited a somewhat higher frequency of thrombocytosis (12%).

CONCLUSION

Thrombocytosis was found in routine examinations of about 5% of hospitalized patients and in a wide range of diseases. If a platelet value in excess of 800 000/μl is observed, a myeloproliferative disorder is the most probable final diagnosis. In cases with moderate thrombocytosis, amyloidosis, rheumatoid arthritis, ankylosing spondylitis, malignancy ulcerative colitis and acute blood loss are common underlying diseases, and should be kept in mind if the clinical picture is atypical.

In a number of diseases the cause of thrombocytosis is obscure and needs further in estigation. However a plasma factor capable of stimulating megakaryocytopoiesis exists (thrombopoietin), and under some conditions it seems probable that immunological mechanisms play a part in the pathogenesis of thrombocytosis.

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Acta med. scand. Vol. 193 pp. 437–446, 1973

CARDIOVASCULAR FUNCTION IN EXTREME OBESITY

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Abstract. Cardiovascular function in 19 patients with obesity has been studied by electrocardiography (ECG), exercise test, determination of heart and blood volumes, and right heart catheterization. Pathological ECG findings appeared in one patient at rest and during exercise, and in another only during exercise. Blood volume was low in relation to body weight, but hemoglobin concentration was normal. Heart volume and stroke volume were normally related to the total amount of hemoglobin (THb), while the working capacity (W_{max}) in eight cases was low in relation to THb. The cardiac output was found to be normal when related to the oxygen intake both at rest and during exercise. There was a statistically significant linear relationship between intravascular pressures and cardiac output. Brachial artery pressures and left ventricle filling pressure were significantly elevated at rest and during exercise, while pulmonary artery pressures and end-diastolic pressure of the right ventricle showed a statistically significant steeper rise with increase in cardiac output than normally found. It is concluded that the circulatory dimensions and the cardiac output reflect an adequate adaptation to the metabolic demands laid upon the circulation in obesity. The elevated filling pressures of the ventricles and the high systemic and pulmonary vascular resistance, however, suggest that obesity is not without deleterious effect on cardiovascular function.

It is widely accepted that obesity induces cardiovascular disturbances, such as prevalence of high blood pressure (for review see 12), and in far advanced cases also congestive heart failure (2, 4, 5, 9). In contrast to the number of clinical reports there are surprisingly few hemodynamic studies of obese patients. Some data obtained at rest during heart catheterization have been presented in case reports (16, 17, 29). Blood volume and cardiac output at rest of 40 obese subjects were determined by Alexander (2), who in the same material measured regional blood flow to the cerebral, splanchnic and renal vascular beds in a few subjects. Pulmonary arterial and pulmonary capillary venous pressures of the same patients were later commented on (1), but not until recently has the central hemodynamic function of a small obese material been described in greater detail (3). The data of the central vascular and intracardiac pressures are, however, rather incomplete and refer to eight and two patients, respectively. Furthermore, most of the data are obtained at rest, and when exercise is included in the investigation procedure, only a single load and a very low one is chosen, provoking an average oxygen consumption of not more than three times the resting value.

The present paper is one in a series of investigations concerning the effect on cardiopulmonary function of weight reduction through jejunoileostomy. The preoperative study of circulatory dimensions and central hemodynamics will be presented below.

MATERIAL

Five male and 14 female patients, 17–59 years of age and weighing 108–172 kg, were investigated prior to jejunoileostomy. They had been obese for several years and treatment with calory intake reduction supported by hospitalization had been of only temporary success.

Fifteen patients had no other diagnosis than obesity while the remaining four had additional diseases. Three subjects were treated for an arterial hypertension and one of them (aged 59) was also taking digitalis and had a typical history of angina pectoris. The fourth patient had a mild, tablet-treated diabetes.

Vital characteristics are presented in Table I.

Table I *Vital statistics of 19 obese patients. Work test data from sitting position*

Case no	Age (y)	Height (cm)	Weight (kg)	Over-weight ( )	BV (l)	W_{170} (kpm/min)	W_{BP} (kpm/min)	HR_{BP} (beats/min)
Males								
1	34	179	170	137	6.8	360	300	162
2	33	184	167	96	7.9	990	900	164
3	44	189	146	85	8.8	1 650	1 200	152
4	34	175	125	55	7.0	1 230	1 200	167
5	47	182	140	63	7.3	1 495	1 000	140
Mean	38	182	149.6	87.4	7.6	1 145	920	157
S.D.	6.6	5.3	18.9	32.3	0.81	506	370	11.0
Range	33–47	175–189	125.0–170.0	55 1 137.0	6.8–8.8	360–1 650	300–1 200	140–167
Females								
6	17	179	143	93	6.2	300	265	172
7	31	158	115	106	5.5	600	600	170
8[a]	35	156	129	119	5.9	1 000	465	144
9	34	167	154	130	8.3	935	800	160
10[b]	32	172	109	44	6.7	690	635	166
11	26	155	108	80	5.8	810	665	161
12	45	170	122	73	6.1	920	600	145
13	42	174	150	—	7.0	1 090	600	138
14[b]	59	171	131	83	7.3	—	150	132
15	32	176	136	76	6.4	655	600	165
16	46	163	141	107	7.4	1 150	465	124
17	27	170	153	120	7.9	890	600	145
18	43	176	172	114	7.7	780	600	152
19	47	162	158	133	7.5	—	—	—
Mean	38	168	137.3	98.1	6.8	818	542	152
S.D.	11.4	7.8	19.4	26.1	0.87	225.0	171.4	15.2
Range	17 59	155–179	108.0–172.0	43.8–133.0	5.5–8.3	300–1 150	150–800	1 4–172

Clinical diagnosis: mild diabetes, [b] arterial hypertension, angina pectoris + digitalis treatment.

METHODS

Before heart catheterization the patients were subjected to a thorough investigation at the laboratory including ECG recordings, exercise tests and determination of total amount of hemoglobin, blood volume and heart volume.

Ideal weight was calculated from height and a measure of skeletal frame, the sum of the wrist widths (13, 14). Overweight is expressed as excess weight as % of the ideal weight.

ECGs were recorded with a direct-writing ink-jet recorder (Mingograf 61 and 81, Elema), both at rest in the supine and standing position and during and after sitting and supine exercise.

The exercise test consisted of stepwise increased work loads (25, 27) on an electrodynamically braked bicycle ergometer (Elema). The loads were increased every 6th min until the subjects were exhausted. The work load at break point, (W_{BP}), was taken to be the highest load at which the subjects worked for 6 min with an increment proportional to the completed period at the next higher load. Physical working capacity (25, 27) is expressed as the working intensity in kpm/min corresponding to a heart rate of 170 beats/min (W_{170}) obtained by intra- or extrapolation.

The heart volume (HV) was measured in the prone position (21).

The total amount of hemoglobin (THb) was determined by the alveolar method of Sjöstrand (26, 27).

The blood volume (BV) was calculated from THb and Hb concentration in finger blood with a standard correction factor (0.91) for the difference between total body hematocrit and the hematocrit of peripheral blood (19).

Right heart catheterization was performed by percutaneous insertion of a double lumen catheter into the left antecubital vein. In the brachial artery of the opposite arm a short teflon catheter was inserted percutaneously.

Blood pressures were measured with strain-gauge transducers (Medec) and recorded, together with an ECG lead, on a UV recorder (Oscilloscript, Siemens). The reference point was taken as the midthoracic point of the anteroposterior plane at the insertion of the fourth rib of the sternum.

Heart rate (HR) was determined from ECG.

The cardiac output was measured according to the direct Fick method. Oxygen uptake was determined by the Douglas bag technique, expired air being measured with a spirometer and gas analyses were made by the Haldane technique. Blood samples were drawn during 1 min from the pulmonary and brachial arteries simultaneously. The oxygen saturation was determined spectrophotometrically and the oxygen content was calculated

from the oxygen saturation and Hb concentration, using the factor 1.34 for the oxygen-combining power of Hb. A correction for physically dissolved oxygen was made according to Peters and van Slyke.

The pulmonary resistance index was calculated as the difference between the mean pressure in the pulmonary artery and the mean pulmonary capillary venous (PVC) pressure divided by the cardiac output; and the systemic resistance index as the mean pressure in the brachial artery divided by the cardiac output.

Current statistical methods have been applied (28). Comparisons between two regression lines have been made according to Hald (18).

The catheterization commenced in the morning after light meal. All measurements were made at rest and during supine exercise at tw loads. The work loads were chosen, with the guidance of the previous exercise tests, to correspond to one submaximal and one close to maximal (W_{HR}) work intensity

RESULTS

Individual data on *blood volume* are given in Table I. Expressed in ml/kg body weight the mean value for the males was 51.1 (S.D. 7.9) and for females 50.5 (S.D 5 0), which are lower values than observed in healthy subjects (74 and 73 ml, respectively) (27), and there was a negative correlation between percentage overweight and BV/kg b. wt. ($p<0.05$). If the BV is expressed in ml/kg ideal b. wt. mean values were 94.3 (S.D 10.5) and 99.2 (S.D 12.3). A positive relationship between oxygen uptake at rest and BV was, however found in this material ($p<0.001$).

Hemoglobin concentration was normal and hence the THb (Fig. 1) aried in accordance with the BV

The values of *heart volume* must probably be regarded as somewhat approximate due to difficulties in getting satisfactory X-ray projections of the cardiac borderlines, caused by the amount of adipose tissue. The absolute values of HV averaged 1 208 ml for the males (range 1 065–1 330) and 976 ml for the females (range 675–1 325). When related to THb four cases were above, one below and the remaining 12 patients within normal limits (Fig. 1).

ECG recordings at rest were normal in 14 patients. Three cases had entricular ectopic beats, which, however vanished during exercise. Pathological left entricle ST T depressions appeared in one case (no. 14 Table I) and slight such changes in another

During exercise pathological ST depressions

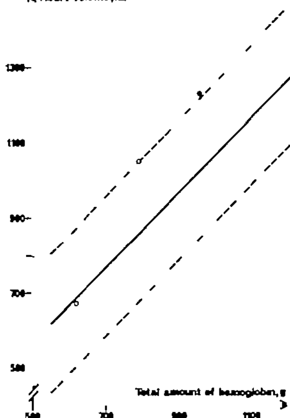


Fig. 1. Heart volume in supine position in relation to total amount of hemoglobin. •, Males ($n=3$); ○, females ($n=14$). Regression line ±2 S.D. for healthy controls are given (27).

corresponding to the left entricle were demonstrated in case 14 (Table I) and in one patient (no. 12) when working in the supine but not in the sitting position. In another case (no. 10) ECG during supine exercise demonstrated slight ST depressions, while no such changes were recorded during sitting exercise. No patients had symptoms of coronary insufficiency during the work tests.

Individual and mean data on *physical working capacity* in the sitting position are given in Table I. The W_{HR}, representing the work actually performed, is lower than W_{170} as most patients discontinued the exercise test before reaching HR 170. The maximal HR, recorded at the end of work, is listed as HR_{HR}. The usual complaint during the final part of the test was a sensation of fatigue in the legs and/or breathlessness.

The W_{HR} was on an average 80 kpm/min higher in the sitting than in the supine position

Table II. *Circulatory data obtained during heart catheterization at rest and during supine exercise*

	Oxygen uptake (ml STPD/min)	A V oxygen diff. (ml/l)	Cardiac output (l/min)	HR (beats/min)	SV (ml)
Rest					
Mean	368	45.7	8.2	75	109
±S.D	54.0	7.42	1.73	9.7	18.6
Range	257–461	34.3–60.1	5.0–11.9	60–97	83–149
	19	19	19	19	19
Load I (100–600 kpm/min)					
Mean	1 286	83.0	15.4	116	133
±S.D	299.5	12.51	2.81	12.1	24.2
Range	750–1 712	66.7 106.4	11.3–22.0	94–137	103–190
	19	19	19	19	19
Load II (300–900 kpm/min)					
Mean	1 816	100.6	17.8	137	131
±S.D	387.2	11.74	2.92	18.4	22.2
Range	1 384–2 478	81.4–113.2	14.4–21.8	110–169	96–172
	17	15	15	17	15

($p < 0.05$) and the patients also broke off the test at a higher HR when sitting, the mean difference being 12.6 beats/min ($p < 0.01$).

W_{170}, which reflects the relationship between load and steady state HR, was on an average 140 kpm/min lower in the sitting position ($p < 0.05$), so on identical loads these patients usually worked with a lower HR in the supine than in the sitting position. When W_{170} (in the sitting position) was related to THb, it was within normal limits in nine cases but below normal in eight. In six of the latter W_{170} was also low in relation to heart volume.

Catheterization data obtained at rest and during exercise are presented in Tables II and III and Figs. –7. The relative physiological stress during work, expressed as percentage of HR_{SF} recorded previously was for the highest work load 97.8%.

The mean data on *cardiac output* are given in Table II. Cardiac output was linearly related to oxygen uptake at rest ($p < 0.01$) and during exercise ($p < 0.001$), and there was no significant difference in regression lines between patients with obesity and ordinary subjects. Individual values are plotted against oxygen uptake in Fig. 2. At rest the cardiac output was increased in two patients and low in another. During exercise—when, in all cases but four two determinations were made—one of those measurements demonstrated high values in three individuals. The remaining 31 observations were normal in relation to oxygen uptake.

Average data on *stroke volume* (SV) at rest and during exercise will be found in Table II. The mean increase on transition from rest to work was 22% ($p < 0.01$), which is slightly higher than in normal individuals, who averaged 13% (15). The variation is wide, however and among the obese subjects even a slight decrease was observed in three cases. During continued exercise the SV remained unchanged.

SV during exercise is related to THb and HV in Figs. 3 and 4. A large SV in relation to THb was found in one patient (no. 9), while two had small SV (nos. 1 and 14). The relation to HV demonstrates a rather wide scatter of the values, probably due to the error in HV determination. SV was large in two patients (nos. 9 and 17) and one had an upper borderline value (no. 16). Five patients (nos. 1 13, 14 15 and 17) had small SV in relation to HV. In cases 15 and 17 however one of the determinations was within normal limits.

During exercise the *pressures* attained higher values in the obese patients than in healthy controls (Figs. 5–8). The difference in regression coefficients was statistically significant ($p < 0.001$) for right ventricle pressures and for pulmonary artery pressures (Figs. 5–6), but there was no difference between the two groups in the slope of the regression lines of PCV pressure and brachial

Table III. *Pressures (mmHg) at rest and during supine exercise*

RV = right ventricle, PA = pulmonary artery, PCV = pulmonary capillary vein, BrA = brachial artery, S = systolic, D = diastolic, De = end-diastolic, M = mean pressure

	RV_S	RV_{De}	PA	PA_D	PA_M	PCV	BrA_S	BrA_D	BrA_M
Rest									
Mean	.8	9	25	13	18	12	143	81	106
± S.D.	0.1	2.8	3.9	3.0	3.4	3.6	22.1	11.5	16.1
Range	17–32	2–14	16–31	9–19	11 23	6–19	112 202	59–103	77–142
	17	17	19	19	19	19	19	19	19
Load I									
Mean	47	12	43	23	32	21	184	94	126
± S.D.	7.9	4.1	10.2	6.6	8.2	7.3	29.3	12.0	22 3
Range	37–59	4–16	29–60	10–36	18–47	7–32	149–272	77–127	101 193
	6[a]	6[a]	18	18	18	17	17	17	16
Load II									
Mean	55	12	48	27	34	21	196	99	133
± S.D.	10.5	3.6	7 7	4.4	7 1	6.9	28.4	11 1	15.3
Range	39–67	4–21	38–70	21 37	21–50	8–31	153–257	77–121	99–167
	14	14	13	13	15	11	17	17	17

Catheter not withdrawn from PCV position in the remaining cases.

artery pressure on cardiac output (Figs. 7–8). However when testing the distance between the two lines, statistically significant differences ($p < 0.001$) were also found for PCV pressure and brachial artery systolic, mean and diastolic pressures.

The difference between pulmonary artery mean pressure and PCV pressure increased linearly with cardiac output ($p < 0.001$). This rise was more marked in the obese group than in healthy subjects ($p < 0.05$), and the mean fall in pulmonary vascular resistance index from 0.81 at rest to 0.75 during exercise was less than in healthy controls.

DISCUSSION

Obesity is defined as overweight due to an increase mainly in the amount of adipose tissue. Fat is an important and active tissue from a metabolic point of view. In obese subjects, for instance, the basic metabolic rate is positively correlated to total body weight (30). The basal blood flow in subcutaneous adipose tissue is of the same order as in resting skeletal muscle and, at least under experimental conditions, the flow capacity may be increased considerably (22, 31). In obesity the total blood flow to fat depots is considered to form an important fraction of the cardiac output, contributing to the reported high resting output of obese subjects (2).

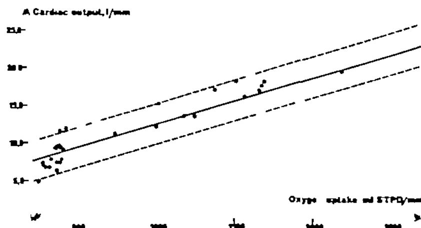


Fig. 2. Relation between cardiac output and oxygen uptake at rest (n = 19) and during supine exercise (n = 34). Symbols and regression line as in Fig. 1.

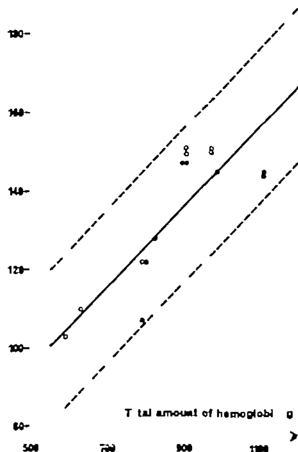


Fig. 3. Stroke volume during supine exercise in relation to THb (= 34). Symbols and normal relationship as in Fig. 1

The circulation adapts to this extra load by increasing its dimensions. It has been shown that with increasing body weight there is an increase in BV and HV (2, 10, 11 24). If these changes reflect a physiological accommodation, they should follow the same interrelations as for healthy individuals of both sexes of varying body size and fitness.

In this study normal relations between THb (in this material equivalent to BV), HV and SV were demonstrated in the majority of subjects (Figs. 1 3 and 4).

W_{170}, which is an indirect measure of the aerobic capacity of the circulation, is low in about half the cases when related to THb and HV. In subjects with normal body size this would be explained by either 1) low SV due to orthostatic reaction or 2) hyperkinetic circulation with ordinary SV or 3) a normokinetic or even hypokinetic circulation with a low SV suggesting impaired heart function.

The first explanation may be applied to two cases: patient 15 whose W_{170} increased from 655 kpm/min in sitting position to 1 065 kpm/min in the supine, which is normal in relation to THb and HV and patient 1 who showed an increase from 360 to 1 115 kpm/min, which is normal in relation to THb but low when related to HV. This is probably due to physical inactivity as his extreme overweight obviously limits his mobility (Table I). Accordingly he had low SV but otherwise normal hemodynamics.

The second explanation, hyperkinetic circulation, suits four cases with low W_{170}, namely nos. 2, 9 17 and possibly 18, who all have a normal SV and a high or somewhat high cardiac output in relation to oxygen uptake.

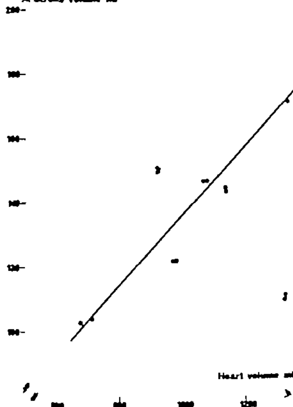


Fig. 4. Stroke volume during supine exercise in relation to heart olume at rest in the supine position (= 32). Symbols and regression line as in Fig. 1.

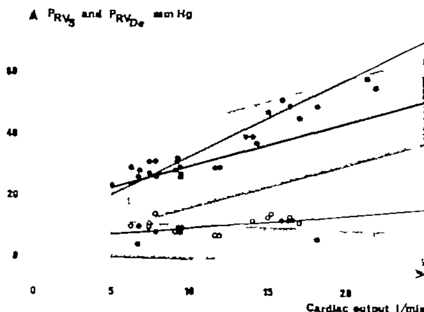


Fig. 5 Relation between right entricle systolic, Pa ( ), and end-diastolic pressure, Pa (O), and cardiac output at rest and during exercise in the supine position (*n* = 36). Full heavy line and shaded area represent the regression ± 2 S.D. found in ordinary subjects (7 15, 20). Full thin line indicates regression for obese subjects.

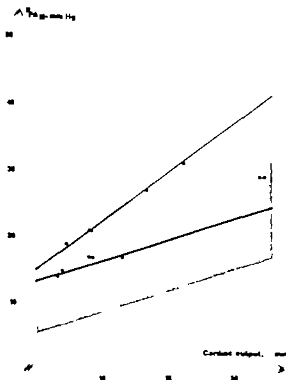


Fig. 6. Pulmonary artery mean pressure, Pa, in relation to cardiac output at rest and during exercise (*n* = 50). Regression lines as in Fig. 5.

Thirdly in four patients, nos. 5, 10, 13 and 14 the reason may be an impaired heart function. Patients 5 13 and 14 have a large HV small SV and filling pressures of the left entricle which were among the highest obtained in these patients. The fourth patient, no. 10 had a normal SV but myocardial insufficiency is likely as she also had an elevated filling pressure. Besides, the most elevated arterial pressures were found in cases 5 10 and 14 in the two latter thus confirming the clinical diagnosis of hypertension. In patients 14 and 10 left entricle dysfunction was also revealed by a pathological ECG reaction during work.

It cannot be ruled out that the heart olumes to some degree are overestimated due, as already mentioned, to an increased error of the method owing to inclusion of epicardial fat in the olume. On the other hand no bias is obvious when HV is related to THb, and the importance of this error which is emphasized by some authors (24), is considered less important by others (5 10, 11).

The normal relationship between oxygen uptake and cardiac output found in this study both at rest and during exercise means that there is an ordinary adjustment of the cardiac output to the oxygen demands.

In a study by Alexander (1) on obese patients of comparable overweight the assumed relation between cardiac output and oxygen consumption

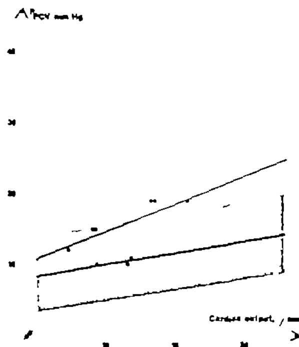


Fig. 7. Pulmonary capillary venous mean pressure, P_{PCV} in relation to cardiac output at rest and during exercise ($n=46$). Regression lines as in Fig. 5

at rest followed approximately the same regression as in our patients, and the arteriovenous oxygen difference was almost the same.

The relation between arterial blood pressure and body weight has been emphasized in a review by Chiang et al. (12). To assess a true increase in systemic vascular resistance, the cardiac output must also be considered. The evaluated systemic arterial pressures found in this investigation indicate a high peripheral resistance, but still a reduction of resistance occurs when flow is increased by muscular work, as the slopes of the regression lines of obese and healthy subjects cannot be distinguished (Fig. 8).

The higher incidence of arterial hypertension among obese individuals is thus verified even if the cardiac output is taken into account.

As regards pulmonary vascular resistance there was, on the contrary, a normal resistance at rest but an impaired reduction of resistance during exercise, which contributes to the pulmonary hypertension found mainly during work. The elevated mean pressure in the pulmonary artery is, however, also due to a high PCV pressure, i.e. high filling pressure of the left ventricle (Fig. 7). As already mentioned, myocardial insufficiency can, at least partly, explain the high filling pressures of the left ventricle in three subjects. In the majority of cases who have elevated filling pressures of both ventricles, other findings suggesting myocardial insufficiency are lacking.

Important factors that affect the end-diastolic pressure are the elastic properties of the ventricular wall and the resistance to ventricular ejection (8, 23). It is possible that, in these patients with large absolute heart volumes and elevated arterial pressures, decreased ventricular compliance and increased systemic vascular resistance can induce high ventricular filling pressures even in the absence of decreased contractility.

High filling pressures in relation to cardiac output have earlier been found in male athletes (6). Compared to them the five obese males of the present study have THb and total BV of the

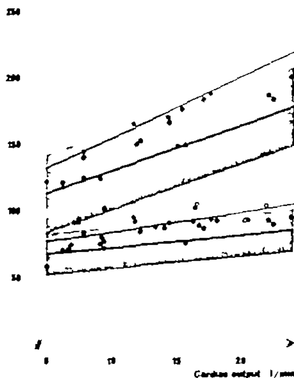


Fig. 8. Brachial artery systolic, P_{BrA_S} (●), and diastolic pressure, P_{BrA_D} (○), in relation to cardiac output at rest and during exercise ($n=52$). Regression lines as in Fig. 5.

same order The range of SV during exercise was for the athletes 149–180 ml and for the obese males 112–172 ml. There is no difference at rest in filling pressure of the left ventricle between the obese males and the athletes, but during exercise two obese males seem to follow the same relation to cardiac output as the athletes and three show a marked increase in filling pressure.

Increased circulatory dimensions can thus, per se, contribute to the raised filling pressures, but it is likely that decreased ventricular compliance and/or impending heart failure are the main reasons. It has also recently been found that, although weight reduction in patients with obesity is accompanied by decreased blood and heart volumes, the filling pressure of the left ventricle remains unchanged (3).

In conclusion, the present study demonstrates, in the majority of the patients, an adequate accommodation of circulatory dimensions and cardiac output to the metabolic demands. The hemodynamic findings, however reveal increased systemic and pulmonary vascular resistance, which brings about an increased load on the central circulation and may lead to impaired heart function.

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Acta med. scand. Vol. 193, pp. 447–456, 1973

LIPOPROTEIN LIPASE ACTIVITY OF HUMAN ADIPOSE TISSUE IN DIFFERENT TYPES OF HYPERLIPIDEMIA

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Abstract. Lipoprotein lipase activity (LPLA) of subcutaneous adipose tissue has been assayed in 249 subjects, who have been grouped according to the electrophoretic pattern of serum lipoproteins or according to a classification based on serum triglyceride and cholesterol determinations with groups corresponding to the electrophoretic types. Type I was not represented in the material. Type IV and group 4 as well as the only three cases with type III are characterized by a lower LPLA per wet weight of the tissue than the serum lipid normal group. Subjects with type II A or group 2 A have about normal LPLA levels, while those with group 2 B seem to have an intermediate LPLA level. The estimated total body content of adipose tissue LPLA was about the same in the different groups, while this estimate as well as the tissue concentration of the enzyme was higher for women than for men in the lipid normal groups. Increasing degree of obesity was correlated with decreasing LPLA, while age per se does not seem to influence the activity level. There was a negative correlation between LPLA and the serum triglyceride level, but the dependence of this correlation on other variables seems to differ between the sexes.

Elevated serum levels of cholesterol and triglycerides have now been widely accepted as risk factors for the development of atherosclerotic diseases (7, 20, 35). Consequently much research has been done to reveal the causes of these abnormal serum lipid levels, but most problems in this field still remain unsolved (13, 29).

Any hyperlipidemia is the result of a disturbed balance between the transport of the lipid into and away from the blood. The mechanisms of the transport of cholesterol and triglycerides are complex and interrelated, as both lipids are constituents of the lipoproteins. However certain transport mechanisms primarily engage a specific lipid component. Thus the enzyme lipoprotein lipase participates in the transport of the triglycerides from the blood to the tissues (29). Adipose tissue derives most of its triglyceride fatty acids from the serum triglycerides (18), a process involving lipoprotein lipase, which is abundant in this tissue. Conversely it is probable that for most subjects adipose tissue is the main assimilation site for the serum triglycerides, even if the muscles may be the site in well trained subjects (29).

A low activity of adipose tissue lipoprotein lipase might play a role in the development of elevated serum triglyceride levels. Our previous findings of a negative correlation between the serum triglyceride level and adipose tissue lipoprotein lipase activity (LPLA) support such a hypothesis (26, 27). To gain further knowledge on these matters, this report deals with measurement of LPLA as well as several blood constituents in a number of normo- and hyperlipidemic subjects, who have been grouped according to sex and blood lipids.

MATERIAL AND METHODS

Three-hundred and thirty-seven individuals have been investigated in the period 1965–71. Eighty-eight of them had special conditions (uremia, diabetes, etc.), which will be dealt with in an accompanying paper (25). The remaining subjects were healthy volunteers and patients of different sex, age and professions. Most of them were investigated on an ambulatory basis, but some were admitted to hospital for investigations of cardiovascular diseases, obesity and hyperlipidemia. All were free from inflammatory and neoplastic diseases and they were not in the acute phase of any cardiovascular disease as judged by interviews and physical and laboratory examinations. Furthermore, in this study subjects with fasting glucose > 150 mg/100 ml, serum creatinine > 3 mg/100 ml as well as those with toxic diseases or un-

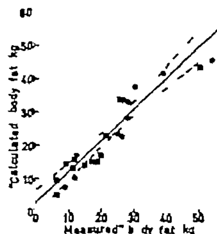


Fig. 1. Linear regression of body fat, calculated from either of the formulas given under Methods, (*y* axis) on body fat estimated by an isotope dilution technique (*x* axis). Estimations by both methods were performed on 25 subjects. – – = 95% confidence limits of $\bar{y}/x$, ■ = men, ● = women.

controlled endocrinological diseases were not included. According to interviews the diet of the subjects showed no major deviation from an ordinary Swedish diet (3). Some patients were on standard hospital diet (2 200 kcal). Subjects on caloric restriction or on diets and medications intended to reduce serum lipids, as well as those with admitted or otherwise known heavy consumption of alcoholic beverages (equivalent to 0.37 l of about 40% (v/v) ethyl alcohol or more per week), have not been included.

After this selection two main clinical groups remained according to the presence (n = 58) or not (n = 191) of atherosclerotic manifestations. As will be shown in an accompanying paper (25), these two groups did not differ much from each other in LPLA. Thus in this report, dealing primarily with comparison between serum lipid groups, the above mentioned two clinical groups will be treated together.

Investigation of the subjects was performed between 7.30 and 9.00 after an overnight fast and 6–10 hours sleep and with no smoking and minimum of physical exercise in the morning. The investigations consisted of needle biopsies from the subcutaneous adipose tissue of the gluteal region for the determination of LPLA. Methodological details of the biopsy procedure and the lipase determinations have been reported previously (26, 27). Unless otherwise mentioned, the results and discussion about LPLA refer to the eluate method with the activity expressed per wet weight of the tissue. In this method the enzyme is first eluted from the tissue with Ringer's solution containing heparin, buffered to pH 7.7 and the enzyme activity is then determined in the eluate (27). The "eluate" method was used for 325 subjects. An additional direct incubation method of LPLA assay was used in 213 of these cases and as a single method in 12 cases. In this method adipose tissue is directly incubated with triglyceride emulsion as substrate (27).

In conjunction with the biopsy procedure, venous blood samples were drawn for the determination of serum triglycerides (tg) (5) and cholesterol (chol) (8), plasma free fatty acids (FFA) (9), blood glucose (33) and acetoacetate (34) and, during the later part of the study, serum lipoprotein electrophoresis (16, 28). The lipoprotein electrophoresis was performed and typed by Dr A. Gustafson. Body weights and heights were also measured. According to body height an "ideal" weight was found in tables published by Lindberg et al. (21). Based on these data a weight index was calculated according to the formula: measured weight/ideal weight, which gives an estimate of the degree of obesity. In 25 subjects the amount of body fat was determined by isotope dilution methods (22). Furthermore, in all subjects with weight index < 2, body fat was calculated according to the formula described by Isaksson et al. (19), i.e. for men (measured – ideal weight) 0.506 + 10.7 and for women (measured – ideal weight) 0.649 + 14.9. These two methods for body fat estimations have been compared in the present material (Fig. 1). It is evident that a high degree of correlation ($r = 0.94$) exists between the methods. The method based on height and weight determinations has thus been considered adequate for the purpose of this study regarding an approximation of the total adipose tissue LPLA. Such calculation of the total lipolytic capacity seems justified if the LPLA of the gluteal region is representative of the total adipose tissue in the body. The experiments recorded in Table I indicate that the LPLA of adipose tissue of different localities is similar enough to justify an approximative calculation of the total adipose tissue LPLA based on measurements in the gluteal region. Adipose tissue biopsies were obtained at the beginning of surgery for uncomplicated gall bladder disease in two women, 31 and 51 years old.

Grouping of hyperlipidemias

The investigations reported here were started before the typing system according to Fredrickson et al. (13) was available to us. It was, however, considered valuable to arrange a classification of hyperlipidemias to fit as closely as possible to the electrophoretic typing system (2). This was accomplished by studying the serum cholesterol and triglyceride values of 155 electrophoretically typed subjects out of the total series (Table II A). For the more common electrophoretic types it was possible to arrange a lipid-quantitative grouping system (with *lipid groups* designated by Arabic figures as contrasted with

Table I. *Adipose tissue LPLA at different anatomical sites*

	Pat. 1	Pat. 2
Gluteal	2.33	5.93
Abdominal		
Below costal margin	2.41	5.60
MacBurney's point	2.53	5.70
Omental	2.35	5.16
Thigh	4.13	5.30

the *electrophoretic types* designated by Roman figures in this report) according to Table II B, which gives a rough 9% "error" in the typing when compared with the electrophoretic system and when only "errors" between pathological groups are considered. The upper limit of the lipid variables in the normal group was put low enough to ensure that no subjects electrophoretically typed as pathological were grouped as normals in the new system. On the contrary about 19% of the subjects electrophoretically typed as normals belonged to the lipid groups 2 A or 4. Subjects with known type III have not been included in the lipid groups.

Quite recently Carlson and Böttiger (7) used a slightly different grouping system based on serum cholesterol and triglyceride determinations only.

Statistical methods

In the comparison of different groups the Mann–Whitney test and for matched pairs the Wilcoxon test, as described by Siegel (30), have been used. In the comparison, for either men or women, of the normal lipid group with either of four abnormal lipid groups, a p level < 0.01 has been considered to denote a statistically significant difference; but if concordant results were obtained for both men and women, a p level < 0.05 was considered sufficient. For testing of the research hypothesis that LPLA was lower in the abnormal lipid groups than in the normal, one tailed" tests have been used, in all other instances "two tailed" tests (30, 32). The Spearman rank correlation analysis (30) as well as standard parametric methods of correlation and multiple regression analysis have been used (33). Calculations have mainly been performed with an IBM 360/65 computer using the Osiris program system for statistical methods developed at the Institute for Social Research, University of Michigan, 1971.

Table II A. *Serum lipid levels in different electrophoretic types of hyperlipidemia (mean ± S.D.)*

Type	No. of subjects	Serum triglyceride (mM)	Serum cholesterol (mg/100 ml)	Cholesterol / triglyceride · 100
Normal	64	1.16±0.44	229±38	2.4±1.1
II A	22	1.50±0.35	342±50	2.4±0.9
II B	17	3.26±1.18	423±122	1.4±0.4
IV	37	3.00±0.83	254±32	0.85±0.20
V	12	15.1±12.4	608±381	0.60±0.60

Table II B. *Lipid groups according to serum triglyceride and cholesterol levels*

Group	Serum triglyceride (mM)	Cholesterol (mg/100 ml)	Cholesterol / triglyceride · 100
Normals	< 1.7	< 280	Any value
2 A	< 2.0	> 280	> 1.5
2 B	2.0–5.0	> 300	Any value
4	2.0–5.0	< 300	< 1.5
5	> 5.0	Any value	Any value

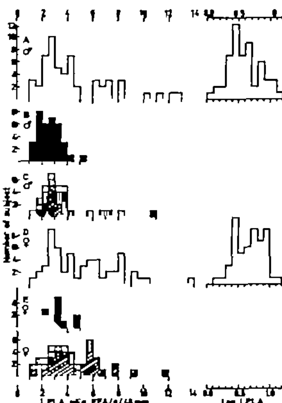


Fig. 2. Distribution of the LPLA and log LPLA variable in men and women. □ = serum lipid normal group, ▨ = group 2 A, ▥ = group 2 B, ■ = group 4, □ = group 5. Groups 2 A, 2 B and 5 appear combined under *C* and *F* in the figure. *A–C* = men, *D–F* = women.

RESULTS

Distribution of variable values

The distribution of LPLA as measured by the eluate method is presented in Fig. 2. LPLA is apparently not normally distributed.

The log LPLA, however, seems to be approximately normally distributed, at least for the men. It is also known that the distribution of serum triglyceride values in a randomly drawn sample of humans is not normal, whereas logarithms of the variable may approximate the normal distribution (6).

Due to the non-normal distribution of these variables, non-parametric statistical methods have been used when possible. However, when a parametric method for correlation analyses has been

Table III. *Adipose tissue LPLA, eluate method (μEq FFA/g/45 min), in different groups of normo- and hyperlipidemia according to serum triglyceride and cholesterol levels as well as in different types according to lipoprotein electrophoresis*

Subjects belonging to some of these types (with the exception of type III) also appear in the lipid groups
Upper rows: mean ± S.D. Middle rows: median within range. Lower rows: no. of subjects and, for the hyperlipidemic groups, significance level in comparison with the normal group

Lipid group	Men	Women	Electrophoretic type	Men	Women
Normal	4.46 ± 2.76 1.09–3.60–12.84 55	5.14 ± 2.63 1.57–4.48–13.60 55	Normal	3.69 ± 2.08 1.09–3.10–11.16 47	4.51 ± 2.47 1.92–3.44–13.60 35
2 A	3.35 ± 2.48 1.65–2.71–10.70 11	4.93 ± 2.28 1.40–4.42–11.78 29	II A	Too few	4.06 ± 1.87 1.81–3.31–7.82 11
2 B	3.80 ± 1.57 1.72–3.75–7.10 13	3.36 ± 1.66 1.05–3.03–5.78 11 ($p < 0.02$)	II B	2.78 ± 0.86 1.72–2.72–4.44 8	Too few
	—	—	III (individual values)	2.22, 2.32	1.92
4	2.55 ± 0.90 0.25–2.44–5.00 36 ($p < 0.0001$)	2.87 ± 1.18 0.61–3.08–4.93 15 ($p < 0.0005$)	IV	2.39 ± 0.81 1.22–2.36–3.79 21 ($p < 0.002$)	2.74 ± 1.16 0.61–2.66–4.43 8 ($p < 0.05$)
5	3.63 ± 2.34 1.32–3.09–8.36 8	3.00 ± 1.19 1.92–2.70–4.78 5 ($p < 0.05$)	V	Too few	Too few

used, as in Table VII, the results fitted in well with the Spearman rank correlation method (30) (unpublished data). This may be due to the use of logarithms of the LPLA and serum triglyceride variables for the parametric method.

LPLA in different lipid groups

The results of the LPLA determinations with the eluate method in different groups and types of hyper- and normo-lipidemic subjects appear in Table III. It is evident that the lipid group 4 as well as type IV is characterized by a lower lipoprotein lipase activity than the corresponding normal group or type.

On the other hand, LPLA seems to be rather similar for group 2 A and the normal group. The number of subjects in groups 2 B and 5 is small and the significance level regarding the comparison between group 2 B and normals for women cannot be regarded as sufficient to demonstrate a clearcut difference between the groups. For the men the median of group 2 B is at about the same level as in the normal group.

The results of determinations with the direct incubation method (Table IV) further confirm that the lipid group 4 has lower LPLA than the corresponding normal group.

When LPLA is calculated so as to be related to an approximation of the total body fat (Table IV), it is evident that there are no differences between the lipid groups. The explanation of this finding is that the men in the hyperlipidemic groups and the women at least in group 4 are more obese than those in the normal group (Table V). An interesting question then arises: is the enzyme concentration of adipose tissue lower in the abnormal lipid groups than in the normal group of equally obese individuals? An attempt has been made to answer that question by matching pairs of subjects in different groups to equal weight index and age without knowledge of other variables during the matching procedure (Table VI). It is evident that also in this situation LPLA is lower in group 4 than in the normal group. In a test of all pair differences between these groups (men + women) p was < 0.001

In a similar test of 21 pair differences (11 men and 10 women) between normals and group 2 B, the latter appeared to have the lower LPLA ($p < 0.05$). Testing between 8 male pairs and 5 female pairs did not reveal any significantly lower LPLA in group 5 than in the normal group, either for the sexes separately or together

No significant differences between the pairs in

Table IV *Adipose tissue LPLA, direct incubation method and total body fat LPLA based on eluate method*

For further explanations see Table III

Lipid group	LPLA, direct incubation method (μEq FFA/g/30 min) Men	Women	Total body fat LPLA, based on eluate method (mEq FFA/body fat/45 min) Men	Women
Normal	9.34 ± 4.59 3.67–8.91 21.3 22	8.71 ± 4.47 3.51 7.[illegible]–21.4 37	44.6 ± 26.5 8.2–37 1–117 55	87.6 ± 57.2 16.8–77.1 354 52
2A	6.08 ± 2.91 3.17–5.57–13.35 9 ($p < 0.05$)	7.81 ± 3.57 2.56–7.34–15.76 21	52.6 ± 28.6 27.9–49.0–126 11	86.7 ± 59.6 21.5–71.1 313 27
2B	5.06 ± 1.72 2.21 5.35–7.38 12 ($p < 0.005$)	6.48 ± 2.59 4.19–5.18–11.64 10 ($p = 0.055$)	58.4 ± 36.1 28.9–48.1 163 13	69.7 ± 62.6 12.2 52.7–220 11
4	5.66 ± 2.07 1.74–5.64–10.07 22 ($p < 0.002$)	6.22 ± 2.13 3.98–6.08–1[illegible].08 12 ($p < 0.01$)	43.2 ± 19.1 8.5–40.8–96.6 36	63.4 ± 41.0 8.2–54.4–157 13
5	7.13 ± 3.54 3.90–5.54–13.57 7	Too few	48.0 ± 21.1 22.0–46.6–80.1 8	61.8 ± 44.7 20.0–46.6–129 5

normal and pathological groups were found for the FFA, aceto-acetate or glucose variables.

Sex differences

Regarding the total body fat LPLA there is an obvious difference between men and women in the normal lipid groups (Table IV $p < 0.0001$), women having higher activity than men. This seems to be due partly to the larger average body fat mass for women (31) (in this series the median for women is 15.5 kg compared with 10.6 for men, $p < 0.0001$ cf also the constants in the formulas for body fat estimation under Methods), but partly also to the higher LPLA

Table V *Average levels of indicated variables in different lipid groups*

Mean ± S.D. (upper rows), median (middle rows) and no. of subjects (lower rows)
The number of subjects may vary due to some missing data for all variables except age

Lipid group	Age (y) Men	Women	Weight index Men	Women	Plasma FFA (μEq/l) Men	Women	Blood aceto-acetate (mg/100 ml) Men	Women	Blood glucose (mg/100 ml) Men	Women
Normal	36.[illegible] ± 15.0	40.3 ± 13.6	1.01 ± 0.13	1.18 ± 0.35	558 ± 197	651 ± 236	0.75 ± 0.37	0.81 ± 0.41	67 ± 9	67 ± 13
	29	40	0.99	1.06	530	614	0.70	0.70	67	65
	57	58	57	58	57	56	52	46	55	54
2A	47.9 ± 9.9	51.4 ± 11.8	1.16 ± 0.15	1.13 ± 0.28	606 ± 160	769 ± 234	0.99 ± 0.40	0.74 ± 0.31	73 ± 11	68 ± 11
	51	53	1.13	1.03	615	783	0.85	0.70	70	67
	11	30	11	29	10	30	8	21	11	28
2B	45.7 ± 7.7	50.2 ± 14.4	1.12 ± 0.16	1.13 ± 0.31	523 ± 166	652 ± 277	0.82 ± 0.22	0.68 ± 0.31	70 ± 8	72 ± 22
	44	51	1.10	1.08	547	568	0.80	0.60	70	71
	13	11	13	11	12	10	10	9	12	11
4	49.2 ± 12.1	44.4 ± 15.6	1.[illegible]1 ± 0.20	1.28 ± 0.30	635 ± 222	677 ± 220	0.66 ± 0.25	0.75 ± 0.34	73 ± 14	78 ± 26
	52	44	1.17	1.25	608	658	0.60	0.60	72	67
	37	16	37	15	37	16	29	11	37	16
5	52.9 ± 10.3	58.4 ± 6.1	1.12 ± 0.15	1.12 ± 0.28	582 ± 212	736 ± 147	0.72 ± 0.17	0.63 ± 0.17	89 ± 18	93 ± 19
	50	58	1.00	0.97	542	691	0.70	0.65	84	80
	8	5	8	5	8	5	6	4	7	5

Table VI. *Comparisons between groups of subjects in a normal group (N) and group 4 (4)*

The groups consist of pairs of subjects matched to each other so that weight index did not differ more than ±0.02 between the individuals. When possible, secondary matching to similar age has been performed. *P* levels in tests of pair differences according to Wilcoxon are given for the LPLA variable

	Men		Women	
	N	4	N	4
LPLA (μEq FFA/g/45 min) (median)	3.68	2.58	4.48	3.09
	$p < 0.01$		$p < 0.025$	
Weight index (median)	1.12	1.12	1.25	1.25
Age (y.) (median)	53	51	46	47
Chol (mg/100 ml) (mean)	230	247	209	252
No. of subjects for variables above	18	18	12	12
FFA (μEq/l) (mean and no. of subjects)	548	666	667	631
	18	18	12	12
Aceto-acetate (mg/100 ml) (median and no. of subj.)	0.65	0.55	0.90	0.60
	16	17	9	10
Glucose (mg/100 ml) (mean and no. of subj.)	67	75	70	68
	18	18	11	11

per gram adipose tissue for the women (Table III) a difference which did not gain statistically significant levels for the whole lipid normal groups of men and women ($p = 0.10$). If however pairs of men and women in the lipid normal group were matched so that the individuals did not differ more than 0.5 kg in estimated body fat, the median LPLA in this group of 27 men was 3.37 and for the 27 women 5.83 μEq FFA/g/45 min. Testing of pair differences yielded significance at $p < 0.001$

Influence of obesity and age on LPLA

It may be anticipated from the results above that the degree of obesity affects the adipose tissue LPLA. This and other relations have been further analysed, as shown in Table VII. At least for the men there is a negative correlation between weight index and log LPLA. It is, however also evident that for men there is a negative correlation between age and log LPLA, while age and weight index are positively correlated. If the weight index variable is kept constant, the partial correlation coefficient between age and log LPLA is 0.096 for men compared with 0.021 for women. The corresponding partial correlation coefficients in the lipid normal subgroups ($n = 55$ for men as well as women) are −0.158 and −0.010. Thus it seems as if age in itself does not influence LPLA much, while increasing obesity as expressed by weight index (or estimated body fat) (unpublished data), is coupled with decreasing LPLA that is, a decreasing concentration of the enzyme in the adipose tissue.

The effect of LPLA and other factors on the serum triglyceride level

There is a negative correlation between log LPLA and log serum triglyceride level for men or women (Table VII)

Table VII. *Correlation matrix of indicated variables for the total series of 125 men (below diagonal) and 114 women (above diagonal)*

n.s. = non-significant correlation

	Log LPLA	Log tg	Chol	Age	Weight index
Log LPLA		−0.383 $p < 0.001$	−0.059 n.s.	−0.044 n.s.	−0.132 n.s.
Log tg	−0.321 $p < 0.001$		0.521 $p < 0.001$	0.373 $p < 0.001$	0.083 n.s.
Chol	−0.113 n.s.	0.746 $p < 0.001$		0.307 $p < 0.001$	−0.094 n.s.
Age	−0.226 $p < 0.05$	0.400 $p < 0.001$	0.207 $p < 0.05$		0.173 n.s.
Weight index	−0.401 $p < 0.001$	0.466 $p < 0.001$	0.265 $p < 0.01$	0.307 $p < 0.001$	

(Rows below the diagonal: Men; above the diagonal: Women)

Table VIII. *Multiple regression with log tg as dependent variable*

Independent variable	*t*-level of regression coefficient			
	Men		Women	
Chol	9.35	Not included in regression	5.24	Not included in regression
Glucose	3.47	4.23	4.60	4.35
Weight index	2.98	4.43	—	−1.06
Age	3.05	3.37	2.06	2.63
log LPLA	−1.19	—	−5.32	−4.80
Aceto-acetate	−1.14	—	—	−3.58
FFA	—	—	−2.46	—
Multiple regression coefficient	0.847	0.676	0.759	0.672
F-ratio for the regression	40.2	27.4	21.2	12.9
No. of subjects	102	102	84	84

There is a difference between men and women regarding the degree of correlation between weight index and log tg, while age in both sexes correlates positively with log tg.

It seemed plausible that the triglycerides contained in lipoproteins relatively rich in cholesterol *might represent a fraction of the serum triglycerides* which were the least accessible to lipoprotein lipase (10, 11) (Tables III and IV). To test this hypothesis, subjects with assumed different levels of cholesterol-rich lipoproteins were separated from each other in an approximate way by an arbitrary serum cholesterol limit, 7 mM.

The coefficient for the correlation between log LPLA and log tg below this cholesterol level was the same for both sexes, −0.482 (81 men and 65 women), while above the same level the negative correlation between log LPLA and log tg disappeared altogether for men but was still negative for women (−0.341 $n = 37$ $p < 0.05$).

With multiple regression analysis an attempt has been made to illustrate the influence of several measured variables on the serum triglyceride level (Table VIII). As may be anticipated from the results in this Table, the correlation coefficient is rather high between glucose and log tg, 0.456 for men and 0.482 for women. Aceto-acetate does not reach any convincingly significant correlation coefficient with any variable except possibly with FFA for women (0.331). FFA has a negative correlation coefficient with log LPLA for men (−0.280) and a positive with weight index for women (0.298). Other correlations were not significant for the FFA and aceto-acetate variables. The partial correlation coefficient between log LPLA and log tg, with the weight index, age, FFA, aceto-acetate and glucose (but *not cholesterol*) *variables kept constant* was −0.056 for men and −0.478 for women.

The *t*-values of Table VIII mainly give a measure of the mutual importance of the independent variables in the regression equations. Variables giving a *t*-value less than 1 have been excluded from the equations. It is evident that for men the log LPLA variable contributes very *little to the multiple regression coefficient*, but that the cholesterol variable dominates very much when present. On the contrary for the women the log LPLA and cholesterol variables seem to be of equal importance and the contribution by the log LPLA variable does not seem to be affected by the additional presence or not of the cholesterol variable.

Average values for age, weight index, FFA, aceto-acetate and glucose in the lipid groups have been given in Table V

DISCUSSION

The lipid quantitative grouping of hyperlipidemias presented under Methods has the advantage of being free from subjective judgement of an electrophoretic pattern. The pathological groups correspond well with the pathological types in the electrophoretic system. The disadvantage of a classification based only on serum lipid determinations is apparently that certain features, as for example the broad beta of type III, will be missed (2). However such conditions are comparatively rare and difficult to diagnose even with lipoprotein electrophoresis (2, 12). Obviously a combination of the electrophoretic system and lipid quantitative aspects on the static classification of hyperlipidemias is preferable in most cases.

These classifications per se do not offer any clues to the etiology of the hyperlipidemias. Nevertheless, they may act as a reference base in the search for etiological mechanisms. Thus it has already been shown that probably the most important defect in the very rare type I is absent or very low LPLA, apparent in adipose tissue (17) as well as in post-heparin plasma (13 14)

Fredrickson et al. (14) did not find any decreased LPLA in post-heparin plasma in other types of hypertriglyceridemia, but recently Boberg (4) with a new technique came to an opposite conclusion.

The present work clearly demonstrates a lower LPLA per wet weight adipose tissue in type IV or group 4 than in the corresponding normal type or group. Subjects with group 2 A have normal LPLA. Those with mixed hyperlipidemias, groups 2 B and 5 seem to have intermediate LPLA levels, while the three identified cases with type III all have low activities. It is possible that unidentified cases with type III occur especially in the lipid group 5

Thus a pattern seems to appear among the hyperlipidemias: subjects with increased serum triglyceride level, but normal or close to normal cholesterol level, have low LPLA, subjects with concurrent increase in the cholesterol level have a LPLA level which is less markedly decreased in comparison with a normal group, while subjects with increases only in the serum cholesterol level have normal LPLA.

There are several differences between the sexes regarding LPLA. Within the lipid normal group the enzyme concentration of adipose tissue as well as the total body content is highest for women (cf ref 24). While increasing obesity is characterized by decreasing LPLA for men, this correlation is less evident for women. It seems, however possible to conclude from the results, especially those in Table VI, that genetic factors also influence the LPLA (cf ref 27).

The results also reveal a difference between men and women regarding correlations between log LPLA and log tg as well as in the multiple regression analysis with log tg as dependent variable. These results might indicate that LPLA would play a more important role for women than for men in the determination of the fasting serum tg level. Besides possible errors in the statistics due to uncontrolled factors as in sampling and variable distributions, there is the possibility of a true difference in this respect. Adipose tissue differs in several qualitative and quantitative ways between the sexes (31). Further lipoprotein lipase in muscular tissue, at least at a young, active age, or other tg assimilation mechanisms may be more important for men than for women (29).

Another possibility which the present results indicate, is that the serum triglyceride, during fasting conditions at high serum cholesterol levels, is a less accessible substrate for LPLA for men than for women. The different lipoprotein composition of male and female sera, especially regarding high density lipoproteins (23), may be of importance in this connection.

In the comparison between pairs of men from a normal group and group 4 matched to each other regarding weight index and age (Table VI), cholesterol was, however at about the same level in the groups. Thus when known or possible biases have been controlled as far as possible, the group with the higher serum triglyceride level is still characterized by a lower LPLA level. In spite of the results with the correlation and multiple regression analysis, it thus seems probable that to some extent a causal relationship may exist between a low LPLA and a high serum triglyceride level even for men.

Evidently a combination of other factors is also active in determining this level for both sexes. Thus in the age factor some active mechanism seems to be disguised, which may not be explained by LPLA in subcutaneous adipose tissue from the gluteal region, but possibly in other tissues, for example the muscles. Other mechanisms which may influence the complex regulation of the serum lipid levels are rate of entry of triglyceride-rich lipoproteins to the blood as well as the rate of catabolism of low density lipoproteins, possible defects in the lecithin cholesterol acyl transferase enzyme system (15) and defects in the LPLA activating system.

It should also be pointed out that the present study has been carried out on subjects after an overnight fast. It may well be that this state does not give the best picture of the lipoprotein lipase system, which presumably assimilates most fat under non-fasting conditions. Further a diurnal variation of the serum triglyceride level in man has been reported (1). Thus when no fat but sucrose was fed during daytime, the highest levels were noted in the mornings at the time when the serum insulin levels were lowest. It may well be argued that the LPLA is not fully stimulated in the mornings due to its dependence on insulin. It may also be argued that many subjects have so low a postabsorptive serum triglyceride level that a substrate induction mechanism of the enzyme may be absent during the night. Such lack

of enzyme induction would also offer an explanation for the variability of the LPLA noted in subjects with low serum triglyceride levels (Fig. 2 and ref 27). On the other hand it seems plausible to assume that hypertriglyceridemic subjects have an adequate enzyme inductive stimulus day and night, but to a variable degree are unresponsive to that stimulus.

ACKNOWLEDGEMENTS

This work was supported by grants from the Swedish Margarine Manufacturers Association, the Swedish Medical Research Council (Project No. B71 19X 2100-7B) and the Mutual Group Life Insurance Company Forenade Liv Stockholm, Sweden.

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Acta med. scand. Vol. 193 pp. 457–462, 1973

LIPOPROTEIN LIPASE ACTIVITY OF HUMAN ADIPOSE TISSUE IN HEALTH AND IN SOME DISEASES WITH HYPERLIPIDEMIA AS A COMMON FEATURE

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Abstract. Adipose tissue lipoprotein lipase activity (LPLA), serum triglycerides, cholesterol and free fatty acids (FFA), blood glucose and aceto-acetate have been determined in groups of 50 healthy men and 50 healthy women. Groups of subjects with atherosclerotic disease, diabetes and impaired glucose tolerance, high alcohol consumption, uremia and some patients with myxedema, nephrosis and myeloma have been studied in the same way. In comparison with majority of normo- and hyperlipidemic subjects the relation between LPLA and serum lipids did not seem to be modified by either atherosclerotic disease or different degrees of glucose intolerance. On the contrary in subjects with alcohol overconsumption LPLA was normal or high, in spite of high incidence of hyperlipidemia. Thirty uremic subjects were characterized by low LPLA, increased levels of serum triglycerides, cholesterol and blood aceto-acetate, while serum FFA was low

Conflicting data are available as to post-heparin plasma lipoprotein lipase activity (PHPLA) in various diseases accompanied by hypertriglyceridemia (2, 3 10, 11 13 15 21 23 24 25). Discrepancies in such findings may be explained by differences in assay techniques as well as in activity pattern of the organs from which PHPLA is released (22). Furthermore, different organs may release *qualitatively different enzyme activities* (6, 12). Due to such circumstances it is preferable to determine lipoprotein lipase activity (LPLA) in one major source of the enzyme such as the adipose tissue. There are, however only few reports on LPLA of this tissue in relation to diseases (8, 9).

In the present study LPLA has been determined in healthy individuals and in subjects with atherosclerotic *manifestations*, diabetes, alcohol over consumption and uremia.

INVESTIGATED SUBJECTS

Healthy volunteers and patients have been studied either on an ambulatory basis or after admission to hospital because of different diseases. They were from the same total series of subjects, as mentioned in preceding paper (19), and they were not in the acute phase of any cardiovascular disease. According to interviews the diet of the subjects showed no major deviation from an ordinary Swedish diet (4). The exceptions were patients with diabetes and some with alcohol overconsumption and nephrosis. Subjects on caloric restriction or on diets and medications intended to reduce serum lipids have not been included.

The series of subjects of the present study has been divided into seven clinical groups without any regard to serum lipid levels:

1. Apparently healthy volunteers: mostly hospital personnel and medical students, investigated without previous knowledge of serum lipid levels. Subjects who were found to have hyperlipidemia were not excluded. According to interviews all were free from acute or chronic diseases.

2. Subjects with atherosclerotic manifestations: myocardial *infarction studied at least 3 months after the* acute episode, typical angina pectoris or claudicatio intermittens.

3. Subjects from group 2 or otherwise healthy subjects with impaired glucose tolerance (20) found at peroral or intravenous glucose loads or with fasting glucose of 86–150 mg/100 ml.

4. Diabetics requiring therapy or subjects with fasting glucose >150 mg/100 ml. Four patients were taking insulin, 11 peroral antidiabetic drugs, and 6 no medication.

5. Alcoholics and *individuals* with an admitted or otherwise known heavy consumption of alcoholic beverages (corresponding to or exceeding 0.37 l week of the standard Swedish "brännvin" containing 40% (v v) ethyl alcohol).

6. Subjects with non-nephrotic uremia (serum creatinine >5 mg/100 ml). All these patients had been referred to the hospital for an investigation in preparation for pos-

Table I. *Distribution of serum lipid groups and average adipose tissue LPLA (mean ± S.D and median) in different clinical states*

Clinical group	Sex	Total no. of subjects	No. of subjects in lipid group Normal	2A	2B	4	5	LPLA (μEq FFA/g/45 min)
Healthy	♂	50	45	2	0	3	0	4.47 ± 2.89 3.31
	♀	50	42	8	0	0	0	5.32 ± 2.55 4.67
Atherosclerotic manifestations	♂	40	11	5	6	16	2	3.33 ± 1.70 3.05
	♀	18	3	7	3	2	3	3.84 ± 1.45 3.97
Impaired glucose tolerance	♂	22	2	5	3	6	6	2.63 ± 0.94 2.56
	♀	16	4	1	3	5	3	3.48 ± 1.47 3.44
"Diabetes	♂	6	0	0	1	4	1	3.32 ± 1.72 3.27
	♀	15	2	1	2	4	6	3.51 ± 2.93 2.63
High alcohol consumption	♂	18	2	0	6	7	3	5.61 ± 2.89 5.09
Uremia	♂	17	7	3	0	6	1	2.45 ± 1.26 2.24
	♀	13	2	8	1	2	0	3.75 ± 1.27 3.46

able future kidney transplantation. None were on dialysis treatment or on protein-restricted diet. Patients with such severe uremic symptoms as would not permit a regular food intake were not included.

7 Patients with special conditions such as myxedema, nephrosis and myeloma.

In groups 2–5 preference has sometimes been given to cases with previously know hyperlipidemia in the selection of subjects for the investigations. The presence in one group excludes the concomittant presence in later group in this order of the groups: 6, 4, 5, 7 2 and 1.

METHODS

Adipose tissue LPLA has been determined according to the eluate method (20). This and other biochemical methods, conditions at the investigations, weight index calculations, grouping of hyperlipidemias and statistical methods have been presented in preceding paper (19).

RESULTS AND DISCUSSION

Pattern of hyperlipidemia in different clinical states

The distribution of individuals between different serum lipid groups in each clinical group has been given in Table I mostly as background information for the discussion of LPLA in these groups. In addition mean and median LPLA values have been tabulated.

The pattern of hyperlipidemia differs between some clinical groups, while it appears similar in the healthy atherosclerotic and uremic groups. On the other hand, men with atherosclerotic manifestations had a higher incidence of hyperlipidemia than reported for a representative sample of men who had sustained a myocardial infarction and were studied from 3 months to 2 years after the acute episode (7).

Average LPLA in healthy subjects

The average LPLA of human adipose tissue as compared with other variables characterizing a healthy group of men and women (group 1) is given in Table II. The weight index of men and women was similar and just below 1.0. A lower average age for the men than for the women may be a result of lack of randomization in the selection of volunteers for the study. Age itself however does not seem to play any important role for the level of LPLA, while at least for men

Table II. *Average values of some variables studied in 50 healthy men and 50 healthy women*

Mean ± S.D. and median within range has been given for each variable

	Men	Women
Age (y)	33.1 ± 13.9 22–26–73	40.7 ± 12.8 17–40–64
Weight index	0.99 ± 0.13 0.78–0.97–1.35	1.01 ± 0.13 0.78–0.96–1.34
Serum triglycerides (mM)	0.99 ± 0.44 0.34–0.98–2.19	0.98 ± 0.44 0.28–0.88–2.52
Serum cholesterol (mg/100 ml)	216 ± 36 140–215–300	229 ± 55 119–214–402
Adipose tissue LPLA (μEq FFA/g/45 min)	4.47 ± 2.89 1.09–3.31–12.84	5.32 ± 2.55 1.95–4.67–13.60
Serum FFA (μEq/l)	548 ± 202 183–521–1037	602 ± 216 225–587–1300
Blood aceto-acetate (mg/100 ml)	0.74 ± 0.38 0.30–0.60–1.90	0.73 ± 0.35 0.30–0.60–1.90
Blood glucose (mg/100 ml)	69 ± 9.0 50–69–91	64 ± 8.3 49–64–80

increasing obesity is correlated with decreasing LPLA (19).

In this report LPLA has been expressed relative to the wet weight of the adipose tissue, which is the most simple and direct way. It seems reasonable to assume that this activity expression of the heparin eluted enzyme may reflect the enzyme concentration at the functional site (22). However, the wide range in the size of the adipose tissue as an organ as well as differences in the total number of fat cells are factors which render an ideal activity expression of the enzyme difficult. It is also possible to relate the activity to total body fat or to a certain number of fat cells, but the functional implications of either of these ways of expressing the activity will be quite different from those of the wet weight related activity (19).

LPLA in relation to serum lipids in some different clinical states

As the selection of subjects for some of the clinical groups has been biased regarding the serum lipid levels, average values of variables

Table III. *Comparison between clinical and control groups*

The total number of men and women in clinical or control group above (A) and below (B) the median of indicated variables of the lipid group to which subject belongs has been counted. Subjects on the median have not been counted. Median values were as reported in Tables III and V in the preceding paper (19) for series of volunteers and patients, who also served as controls in this comparison, when appropriate, after exclusion of subjects belonging to the clinical group compared. Possible differences in the distribution around the above mentioned medians between the clinical and control groups were evaluated by the χ^2-test, and p values < 0.05 are indicated in the Table

	No. of subjects		Adipose tissue LPLA (μEq FFA/g/45 min)		Age (y)		Weight index		Serum FFA (μEq/l)		Blood aceto-acetate (mg/100 ml)		Blood glucose (mg/100 ml)	
	Men	Women	A	B	A	B	A	B	A	B	A	B	A	B
Atherosclerotic manifestations	40	18	29	25	40	12	23	24	32	23	23	18	22	24
					$p < 0.0005$									
Controls	86	102	86	90	77	105	93	92	87	96	68	73	86	84
Impaired glucose tolerance	22	16	16	20	22	13	26	10	21	15	14	10	—	
							$p < 0.005$							
Controls	104	104	99	95	95	104	90	106	98	104	77	81	—	
Diabetes	6	15	8	9	11	9	11	10	10	9	11	3	—	
											p 0.05			
Controls	126	120	115	115	123	123	116	116	119	119	91	91	—	
High alcohol consumption	18	—	14	3	10	8	11	7	8	10	5	8	8	8
			$p < 0.02$											
Controls	126	—	60	60	61	61	60	60	61	61	49	49	54	54

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MUSCLE TRIGLYCERIDES

Relation to Glycogen in Muscle and Plasma Triglycerides in Men of Different Ages

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Abstract. Muscle tissue from the lateral vastus of the femoral muscle was taken by needle biopsy from 39 healthy fasting men 20–61 years of age and analysed for triglycerides, phospholipids and glycogen. The mean muscle triglyceride concentration increased with age and was 10.2 ± 0.9, 12.8 ± 1.6, 15.9 ± 1.0 and 16.2 ± 1.0 μmole/g for men in the third, fourth, fifth and sixth decades, respectively, while no consistent change was found in the mean concentration of phospholipids and glycogen. The concentration of phospholipids and glycogen was higher in subjects who reported "increased amounts of habitual physical activity" than in those reporting "normal" level. The muscle triglyceride concentration was apparently not influenced by the level of "physical activity". The concentration of triglycerides in muscle was positively correlated to age and to the plasma triglyceride concentration. The correlation between the triglyceride concentration in muscle and plasma remained when the influence of age on these variables was eliminated by partial regression analyses. The possibility is discussed that the plasma triglycerides are of importance for the muscle triglyceride metabolism by supplying fatty acids for the triglyceride synthetizing system in the muscle tissue. No correlation was found between the concentration of triglycerides and glycogen in muscle. The standard deviation for differences in the triglyceride concentration between duplicate muscle samples was 2.4 μmole/g for subjects 20–39 years of age, 6.4 μmole/g for subjects above 40 years of age. The corresponding values for the phospholipid concentration were similar in all age groups with a mean of 0.8 mg/g for all subjects.

Recent studies have suggested that the skeletal muscle triglycerides may be a metabolically active lipid fraction during exercise (20, 21, 24). Little is, however, known about what factors regulate their metabolism. Fatty acids are assimilated in muscle triglycerides in vivo (25, 29) and in vitro (1, 15) and there is evidence suggesting that the level of muscle triglycerides is responsive to changes in the flow of plasma FFA (6, 7). Other lines of evidence suggest that the level of muscle triglycerides is influenced also by other factors than acute changes in the availability of plasma FFA. Thus the muscle triglyceride concentration in rat has been reported to increase with age (9) and to be reduced by physical training (10, 19), and in man to be negatively related to work performance (12). Furthermore, in alloxan diabetic rat the muscle triglyceride concentration was increased (13), which suggests that the muscle triglycerides are associated with or are part of insulin-dependent pathways for lipid metabolism.

Muscle is a potent tissue for oxidation of fatty acids and it is likely that the muscle triglycerides serve as a moderator for the internal flow of fatty acids from cell surface to mitochondria in the muscle cells. Jones and Havel (25) demonstrated assimilation of chylomicron fatty acids in muscle lipids. *This suggests that the muscle triglycerides* may assimilate also plasma triglyceride fatty acids, e.g. the metabolism of muscle triglycerides may be related to the metabolism of plasma triglycerides.

In the present study the triglyceride concentration has been determined in muscle tissue obtained by needle biopsy from the lateral vastus of the femoral muscle in men of different ages. The *muscle triglyceride concentration was related both* to age and to the plasma triglyceride concentration. Furthermore, in a previous study we found no immediate relationship between the acute decrease in the concentration of triglycerides and glycogen in muscle during exercise (12). This suggests that the metabolism of the two major muscle stores of substrate was influenced by different mechanisms during exercise. To get some further

Table I. *Age, weight–height index and plasma triglyceride concentration*

	Age interval (y.)			
	20–29	30–39	40–49	50–61
Age (y.)				
M	24.2	34.0	44.1	55.4
S.E.M.	0.53	1.18	0.65	0.96
	19	8	17	15
Index[a]				
M	0.93	0.96	1.05	1.06
S.E.M.	0.02	0.03	0.03	0.03
	19	8	17	15
Plasma TG (mmole/l)				
M	0.94	1.27	1.50	1.30
S.E.M.	0.07	0.16	0.11	0.11
	11	8	15	14

[a] weight/(height – 100).

information on this matter the relationship between the concentration of triglycerides and glycogen in muscle was determined also under basal resting condition.

SUBJECTS AND METHODS

Fifty-nine male subjects participated in the study. All had sedentary occupation. None complained of disease or physical discomfort or admitted to taking any drugs. Laboratory tests to evaluate their clinical status were not done. Some subjects reported physical activity outside of working hours, such as bicycling to work, jogging or participating in sports such as tennis and ball games. These were classified as physically active.

The subjects were divided into four age groups; 20–29, 30–39, 40–49 and 50–61 years of age. Table I gives the mean values for the age, the weight–height index (weight/(height-100)) and the concentration of plasma triglycerides for subjects in each age group.

The subjects reported to the laboratory after an overnight fast. None had been engaged in unusual physical activity or had deviated from their ordinary dietary habits during the 3 days preceding the day of sampling. Blood was collected into heparinized syringes from an antecubital vein. Muscle tissue was taken by needle biopsy technique (2) from the belly of the lateral vastus of the femoral muscle midway between the knee and the trochanter major. Sampling was done between 8 a.m. and 10 a.m. during May and June.

Muscle tissue from both legs was separately analysed for lipid content. Visible adipose tissue and connective tissue were removed by dissection under a magnifying glass (×5). The weight of the muscle sample was determined by extrapolation after weighing three times on an electromagnetic balance. During the third and fourth min after the biopsy when weighing was done, the mean weight loss was 0.414±0.014 (n=80) mg/min. Analysis of muscle lipids was done in principle as described earlier (17). 20–40 mg of the muscle tissue was homogenized in all glass homogenizers with 1 ml of methanol. This procedure was completed not later than 5 min after the biopsy. Two ml of chloroform was then added to the methanol homogenate, followed by 3 ml of saline. The concentration of triglycerides (5) was determined in triplicate and the concentration of phospholipids (4) in duplicate on aliquots of the chloroform phase. 5–10 mg of muscle tissue from one leg only was separated from the specimen obtained in the biopsy needle and used for determination of glycogen (23). The concentration of plasma triglycerides was determined according to Kessler and Lederer (26).

Table II. *Concentration of triglycerides, phospholipids and glycogen in the vastus lateralis of the femoral muscle*

p = degree of significance between group means

	Age interval (y.)				
	20–29	30–39	40–49	50–61	20–61
Triglycerides (μmole/g)					
M	10.2	12.8	13.9	16.2	13.7
S.E.M.	0.9	1.6	1.0	1.0	1.9
n	19	8	17	15	59
p		>0.05	>0.05	>0.05	
Phospholipids (mg/g)					
M	8.1	6.9	8.1	7.3	7.8
S.E.M.	0.3	0.3	0.3	0.3	0.1
	17	8	16	14	55
p		<0.05	<0.05	>0.05	
Glycogen (mg/g)					
M	9.7	9.0	9.5	8.4	9.2
S.E.M.	0.8	0.7	0.7	0.4	0.4
n	14	8	14	14	50
p		>0.05	>0.05	>0.05	

RESULTS

Table I shows that the mean values for the plasma triglyceride concentration and for the weight height index with advancing age followed the same pattern as has previously been reported for healthy men (8).

Table II gives the concentration of triglycerides, phospholipids and glycogen in muscle tissue. The muscle triglyceride concentration increased consistently from a mean value of 10.2 μmole/g in the third decade to 16.2 μmole/g in the sixth decade. The phospholipid concentration remained essentially unchanged through the four decades. For all subjects the mean value was 7.8±0.1 mg/g. The glycogen concentration showed no consistent change with advancing age. The mean

Table III. *Individual differences for the concentration of triglycerides and phospholipids in muscle tissue from the right and the left leg*

	Age interval (y.)				
	20–29	30–39	40–49	50–61	20–61
Triglycerides (µmole/g)					
M	−0.80	−1.06	−1.49	−2.61	−1.50
S.E.M.	0.79	1.21	1.73	2.20	0.79
	19	8	17	15	59
p	>0.05	>0.05	>0.05	>0.05	
S.D.	2.40	2.38	5.02	6.11	4.40
Phospholipids (mg/g)					
M	0.26	0.13	−0.46	−0.19	−0.08
S.E.M.	0.32	0.58	0.37	0.36	0.19
	17	8	16	14	55
p	>0.05	>0.05	>0.05	>0.05	>0.05
S.D.	0.92	1.10	1.07	0.92	0.84

$\sqrt{\Sigma d^2/2N}$

muscle glycogen concentration was 9.2 ± 0.4 mg/g for all subjects.

Subjects reporting "physical activity" tended to have higher values for the muscle concentration of phospholipids and glycogen (Figs. 2 and 3). Since no consistent changes were observed in the concentration of phospholipids and glycogen with age (Table II, Figs. 2 and 3) a mean value was calculated for all subjects reporting "physical activity" and those without. The phospholipid concentration was 8.8 ± 0.2 ($n = 19$) for "active" and 7.3 ± 0.2 mg/g ($n = 36$) for the "non-active" subjects ($p < 0.001$). The corresponding values for the glycogen concentration were 11.1 ± 0.4 ($n = 18$) and 8.1 ± 0.4 mg/g ($n = 32$) respectively ($p < 0.001$).

Table III gives the mean and the S.E.M. for the individual differences of the concentration of triglycerides and phospholipids between the right and left legs. As is seen, the concentration of triglycerides and phospholipids showed no consistent change between legs in any of the age groups or when all subjects were taken together. The S.D. for the difference in the triglyceride concentration was 2.40 and 2.38 µmole/g for the younger age groups and 5.02 and 6.11 µmole/g for the older. The S.D. for the difference in the phospholipid concentration remained essentially unchanged with advancing age. For all subjects the value was 0.84 mg/g or 10.8% of the mean value for the phospholipid concentration (Table II).

TRIGLYCERIDES
µmol

20

Fig. 1. Muscle triglyceride concentration and age. The equation for the regression line of all subjects is $y = 0.22x + 5.1$. The symbols indicate subjects with ( ) and without (O) reported "physical activity".

The values for the absolute difference in the muscle triglyceride concentration between legs was skewly distributed ($g = 1.38$, $p < 0.001$) with a median value of 3.66 and interquartile range from 1.53 to 6.43 µmole/g. The values for the absolute difference in the triglyceride concentration between legs was positively correlated to age ($r = 0.45$, $p < 0.001$). This correlation disappeared when, instead, the logarithmic values for the absolute difference were used. Also the absolute difference between legs for the phospholipid concentration was skewly distributed ($g = 1.82$, $p < 0.001$). The median value was 0.60 mg/g with an interquartile range of 0.30 to 1.37 mg/g. No correlation was found between the difference between legs and age. In Table IV the accumulated values are given for age, the absolute difference for the muscle triglyceride concentration between legs and the S.D. ($\sqrt{\Sigma d^2/2N}$) for the absolute differences through quartile 1 to 4.

Correlation constants for some of the variables are listed in Table V. The triglyceride concentra-

PHOSPHOLIPIDS
mg/g

10

20 … 50 YEARS OF AGE

Fig. 2. Muscle phospholipid concentration and age (symbols as in Fig. 1).

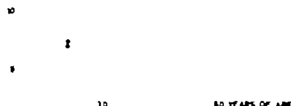


Fig. 3. Muscle glycogen concentration and age (symbols as in Fig. 1).

tion in muscle was positively correlated to age, while no correlation was found between the muscle triglyceride concentration and the weight height index. No correlation was found between age and the concentration of phospholipids or glycogen, nor was there a correlation between the concentration of triglycerides and glycogen in the muscle. Interestingly a positive correlation was found between the triglyceride concentration in muscle and plasma. As is shown in Table V the plasma triglyceride concentration was also correlated to age. Partial regression analysis revealed, however an age-independent correlation, although of low significance level, between the triglyceride concentration in muscle and plasma.

The index of skewness for the muscle triglyceride concentration was 0.33 ($p > 0.05$) with a median value of 13.4 µmole/g. The corresponding values for the plasma triglyceride concentration were 0.57 ($p > 0.05$) and 1.26 mmol/l.

Table IV *Mean of the accumulated values for age, absolute difference between duplicate muscle samples and S.D. of differences for the triglyceride concentration through quartile 1 to 4*

Division into quartiles was done on the basis of the absolute differences in the triglyceride concentration between duplicate muscle samples

Quartile		Age (y.)	Triglycerides (µmole/g) Absolute diff.	S.D.
1	15	35±2.8	0.99±0.28	0.77
1 2	30	34.4±2.0	1.87±0.19	1.51
1 3	45	37.3±2.0	2.92±0.27	2.42
1–4	59	39.4±1.6	4.78±0.53	4.40

Table V *Some correlation constants for some of the investigated variables*

TG_m = triglyceride, PL = phospholipid and Glg = glycogen concentration in muscle, TG_{pl} = triglyceride concentration in plasma, Index = weight-height index

x	y	n		p
Age	TG_m	59	0.60	<0.001
Index	TG_m	59	0.26	>0.05
Age	PL	55	−0.07	>0.05
Age	Glg	50	−0.17	>0.05
TG_m	Glg	50	0.04	>0.05
TG_{pl}	TG_m	48	0.46	<0.005
Age	TG_{pl}	48	0.40	<0.01
Age	TG_m	48	0.56	<0.001
Partial regression				
Age; TG_m	TG_{pl}	48	0.46	<0.01
Age; TG_{pl}	TG_m	48	0.19	>0.05
TG_m TG_{pl}	Age	48	0.31	<0.05

DISCUSSION

No quantitative studies are available on the concentration of triglycerides in skeletal muscle of healthy men of different ages. Studies on muscle lipids have, however been made on autopsy material either by estimating the amount of lipids histologically and/or by determining the total amount of fat (15 16, 19 22, 27). Determined in this way the total amount of fat has been reported to increase with age. Helander (22) found that the total lipid content of gastrocnemius muscle, on a dry weight basis, increased from 12.2 to 21.2% from the third to the sixth decade, mainly due to accumulation of extracellular adipose tissue. Assuming a water content of 75% in the muscle tissue, this corresponds to an increase from about 3 to 5.3% wet weight. In newborns with no or extremely small amounts of extracellular adipose tissue, the corresponding value was 2%. In the present study the triglyceride concentration, determined by a specific method, increased consistently from 0.8 in the younger to 1.4% in the older age groups. The corresponding values for the sum of the mean values of the concentration of triglycerides and phospholipids were 1.5% and 2% respectively. The cholesterol concentration, which was not determined in the present study has been reported to be about 0.3% of the dry weight (14).

One question that needs to be considered when

studying the triglyceride metabolism in muscle is whether the triglycerides determined are derived from extra" or intracellular" sources. From the physiological point of view this may be of importance, since extra- and intracellular triglycerides may have a different role in the lipid metabolism. Therefore an attempt was made to determine the intracellular triglyceride concentration by removing visible extracellular adipose tissue by dissecting the muscle tissue under magnification (× 5) before lipid analysis.

The values for the absolute difference between duplicate muscle samples ranged from 0.12 to 18.6 µmole/g, probably due to varying success in removing extracellular adipose tissue. Further analysis of the data showed that the largest differences tended to occur in the older subjects, as might be expected since extracellular adipose tissue is more abundant in older than in younger subjects (22). The values for the absolute differences were, however skewly distributed towards lower values and their logarithms were not correlated to age. This suggests that the increase in the mean muscle triglyceride concentration with age (Table II, Fig. 1) was not due to accumulation of extracellular adipose tissue alone. Furthermore, the correlation between age and the mean muscle triglyceride concentration remained when only values with a small variation between duplicate muscle samples were used, e.g. from the first ($r = 0.70$, $p < 0.005$) or from the second ($r =$ 0.78 $p < 0.001$) quartile separately.

Rapid changes have been demonstrated both in the mean muscle triglyceride concentration (7 18) and in the amount of sudanophilic material (6) in the muscle fibres with alteration of the plasma FFA flow. Although these and other studies (1 29) indicate that a change in the flow of the plasma FFA have acute effects on the level of muscle triglycerides (6, 7) it is likely that the steady state level is determined by the total flow of fatty acids into the muscle, e.g. by fatty acids derived not only from plasma FFA but also from the triglyceride-rich plasma lipoproteins.

Enzymatic equipment for removal of plasma triglyceride fatty acids (TGFA) (28) as well as extraction of TGFA from plasma has been reported for skeletal muscle (25) and for myocardium (11). Furthermore, ageing increased (9) and physical training decreased the triglyceride concentration in plasma and muscle tissue (10, 19). The correlation between the concentration of triglycerides in muscle and plasma found here may indicate that the plasma triglycerides contribute fatty acids to the triglyceride synthetizing system in muscle tissue. Further studies are, however necessary to evaluate this hypothesis.

No major changes occurred in the concentration of phospholipids and glycogen in muscle with age. The concentration of phospholipids and of glycogen was, however higher in subjects reporting increased amounts of habitual physical activity" Physical training has been reported to elevate the phospholipid concentration (19), probably as a consequence of increased amounts of membranous material in the muscle tissue. The glycogen level in the muscle tissue is, however dependent on the carbohydrate intake (3) and the diet during the days preceding the muscle sampling was not known.

It is of interest that no correlation was found between the concentration of triglycerides and glycogen in the muscle. This suggests that the metabolisms of carbohydrate and fat in the muscle are not directly related to each other a hypothesis that is supported by the lack of relation between the variation in the concentration of triglycerides and glycogen in muscle tissue during exercise (12).

ACKNOWLEDGEMENTS

Supported by grants from the Swedish Medical Research Council (19x-204) and The Swedish Nutritional Research Foundation.

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Acta med. scand. Vol. 193 pp. 469–475 1973

CELL SIZE AND LIPOLYSIS BY HUMAN SUBCUTANEOUS ADIPOSE TISSUE

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Abstract. Specimens of human subcutaneous adipose tissue were removed from subjects with marked difference in body weight, patients with recent onset of diabetes mellitus of juvenile and adult type, and from obese subjects before and after intestinal shunt operation. The production of glycerol by sections of adipose tissue of the non-diabetics under basal (glucose conc. 5.5 mM) as well as stimulated conditions was positively correlated to the mean cell volume estimated according to the histometric procedure. The maximal lipolytic responses to theophylline and $N^6,O^{2'}$-dibutyryl 3'5' AMP expressed as the relative change in the glycerol production above the basal values, were negatively related to the mean cell volume. The addition of the α-adrenergic blocking agent phentolamine increased more markedly the noradrenaline-induced glycerol production by small fat cells than that by enlarged cells. The findings clearly show that the size of the fat cells has a substantial influence on the lipolysis of human fat cells. Adipose tissue of large cells might respond with less marked lipid mobilization than adipose tissue composed of small cells. This could be of relevance for the understanding of the non-ketotic hyperglycemic syndrome.

A number of investigators have shown that the size of the fat cell has a significant influence on the rate of the synthesis as well as the breakdown of the triglycerides in adipose tissue incubated in vitro. However, the relationship between the size of the human fat cell and the rate of lipolysis stimulated by different agents, e.g. catecholamines, seems to be far from clarified. Thus enlarged human adipocytes have been reported to possess decreased (5, 14, 20, 21, 31), unchanged (12, 13) and even increased (14, 25) lipolytic response to either noradrenaline, adrenaline or theophylline when added in vitro and when the lipolytic response to the agent is defined as the relative change in the rate of glycerol release in comparison with the basal values. Almost all mentioned authors investigated the release of glycerol from large and normal-sized fat cells through the use of subcutaneous adipose tissue excised from obese and non-obese donors, respectively. Goldrich and McLoughlin (14) base their conclusions from paired observations on lipolysis by omental and subcutaneous adipose tissue obtained from the same donor, thereby exploiting the fact that the adipocytes of the latter origin are significantly larger.

In the present study the relation between the size of the fat cell and lipolysis was explored in human subcutaneous adipose tissue in two ways, firstly by taking biopsies from subjects with marked differences in body weight and secondly by taking biopsies at intervals from subjects with marked induced changes in body weight. The second situation was made possible in patients with massive obesity by submitting them to small intestinal shunt operations. The adipose tissue obtained was exposed in vitro to agents which stimulate lipolysis apparently at different sites of action: noradrenaline, isoprophylnoradrenaline, dibutyryl cAMP and theophylline.

MATERIAL AND METHODS

Specimens of adipose tissue were removed from the subcutis of the anterior wall at the time of operation of six obese subjects undergoing jejuno-ileostomy and of three patients undergoing cholecystectomy. In the six obese patients adipose tissue was excised also by biopsy at intervals (Table II) when the body weight had decreased after the jejuno-ileostomy. In another seven patients, who had previously been treated with jejuno-ileostomy because of obesity, adipose tissue was removed only by biopsy on one or several occasions. Some clinical data of the 16 subjects included in the study are given in Table I.

Table I. *Clinical data of the patients included in the study*

Figures within parentheses indicate number of biopsies studied, J-I = jejuno-ileostomy

			B. W.		Glucose (i.v.) tolerance	
Pat. no.	Age (y.)	Sex	(kg)	(% of the ideal)	(K) (%/min)	Type of operation
1	17	♀	143	225	0.82	J-I + biopsy (2)
2	37	♂	135	182	0.86	J-I + biopsy (2)
3	18	♀	130	221	1.18	J-I + biopsy (2)
4	40		122	221	1.03	J-I + biopsy (1)
5	44	♀	118	198	1.47	J-I + biopsy (1)
6	43	♀	172	270	1.74	J-I + biopsy (1)
7	24	o	74	106	—	Cholecystectomy
8	28	♀	58	106	—	Cholecystectomy
9	23	♂	87	124	—	Cholecystectomy
10	46	♀	120	202	1.21	Biopsy (2)
11	[illegible]	♀	95	152	0.84	Biopsy (2)
12	[illegible]	♀	131	249	2.18	Biopsy (3)
13	45	♂	109	162	0.95	Biopsy (1)
14	37	♀	140	244	0.47	Biopsy (1)
15	43	♂	93	134	—	Biopsy (1)
16	25	♀	124	222	—	Biopsy (1)

Altogether 29 experiments were undertaken. The ideal body weight was obtained from the tables computed by the Metropolitan Life Insurance Company (24). The i.v. glucose tolerance test (19) was performed in most of the obese subjects.

Surgical procedure

obese patients were treated by two types of intestinal shunts, both preserving 50–70 cm functional intestine. One type was jejuno-ileostomy end-to-side as described by Payne and De Wind (30). The second type was a jejuno-ileostomy end-to-end plus ileo-coecostomy end-to-side, the principle of which was also described by Salmon (32). The abdominal incisions were made transversally from the umbilicus to the right. The procedures were performed under general anesthesia, after fasting overnight, with intubation and using muscle relaxants (succinylcholine). The anesthesia was induced with short-acting barbiturate, Narkotal® (Astra, Södertälje) and continued with Halothan® (Hoechst, Frankfurt, West Germany). Only saline was given until the biopsy was removed. This was done early during the operation when the patient was fully anesthetized. The biopsy was taken from the subcutaneous adipose tissue in the cranial border of the incision. The adipose tissue was immediately transferred into a Krebs-Ringer albumin solution kept at 37°C.

The postoperative biopsies were taken at intervals during the follow-up. All subjects fasted overnight. The biopsies were taken from the subcutaneous adipose tissue from the abdomen on the left side with a small incision. In some individuals several postoperative biopsies were taken. These were all taken from the left side of the abdomen from an area with intact innervation and symmetrically positioned with the first biopsy on the right side of the umbilicus. A local anesthetic was used, Xylocain® 1% (Astra, Södertälje) and injected intradermally as described in detail elsewhere (2). Care was taken not to contaminate the adipose tissue in the biopsy when the local anesthetic agent was used, since apparently this has strongly antilipolytic properties in rat adipose tissue (15) as well as in human adipose tissue (2).

Incubation procedure

The tissues were divided into sections weighing approximately 50 mg each and preincubated for 30 min in Krebs-Henseleit bicarbonate buffer containing 3% bovine serum albumin (Armour Pharm. Co., Eastbourne, Lot number R No. 970) and 100 mg of glucose 100 ml. About 150 mg of adipose tissue was incubated in 3 ml medium solution in polyethylene vials cycling 80/min at 37°C. After two hours of incubation two aliquots (0.1 ml) of the medium were removed for glycerol determination as described by Wieland (36) and modified by Chernick (9). Glycerol production (μmoles/10^6 cells) was calculated as the mean of triplicate incubations corrected for the non-incubated controls.

Adipose tissue was homogenized in glass and the lipids extracted as described by Dole (10). The lipid content of adipose tissue was determined gravimetrically.

Agents added in vitro were: L-noradrenaline bitartrate (supplied by Astra, Södertälje, Sweden), L-isopropylnoradrenaline-d-bitartrate dehydrate (supplied by Dr F. P. Luduena from Sterling Winthrop Research Institute), theophylline, N^6,$O^{2'}$-dibutyryl 3',5'-AMP and phentolamine HCl (supplied by Ciba, Stockholm, Sweden).

All agents except theophylline were dissolved in distilled water; 0.1 ml of this was added to the incubation medium. Theophylline was directly dissolved in the albumin-containing buffer. From a great number of earlier experiments it was ascertained that each lipolytic drug was used in a concentration which induced maximal

lipolysis (noradrenaline, 2 10[illegible] M/ isopropylnoradrenaline, 2 10[illegible] M/ dibutyryl cAMP 1 10[illegible] M/ and theophylline, 1 10[illegible] M/). Phentolamine HCl was added at two concentrations, 0.5 and 50 μg/ml, to noradrenaline-containing medium.

Cell size determination

Two fat specimens weighing about 20–30 mg each were used for the determination of the cell diameter according to the procedure developed by Sjöström et al. (33). The diameters of 100 cells were measured with calibrated ocular in Zeiss photomicroscope. Observer bias appeared to be insignificant. The mean cell volume was calculated from the average diameter and the S.D. of the diameter according to the formula discussed by Hirsch and Gallian (18) and Nestel et al. (26). The number of fat cells incubated was calculated using the mean cellular triglyceride content and the total triglyceride content of the fat portions.

The statistical calculations were performed as described by Snedecor (35).

RESULTS

Fig. 1 shows that significant correlation existed between the mean volume of the fat cell and the body weight, expressed in percentage of the ideal ($r = +0.69$, $p < 0.001$). The mean volume of the

Table II. *Effect of jejuno-ileostomy on the body weight and the size of fat cells in subcutis*

Pat. no.	Time between biopsies (mo)	B.wt. (kg)	B.wt. (% of the ideal)	Average diameter (μ)	Mean cell volume (mm³ 10[illegible])
1	—	143	225	129	1 185
	3	124	195	105	766
	6	96	144	86	400
2	—	135	182	122	1 015
	2.5	107	144	110	745
	5	92	124	90	431
3	—	130	221	142	1 584
	2.5	121	206	126	1 143
	8.5	105.5	177	117	902
4	—	122	221	126	1 152
	3	100	181	102	613
5	—	118	198	120	920
	2.5	107.5	181	99	515
6	—	172	270	134	1 378
	1.5	155	243	126	1 144

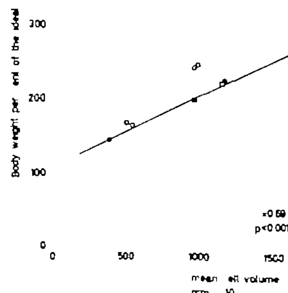


Fig. 1. Relation between the mean cell volume of fat cells and the body weight, as percentage of the calculated ideal weight (see Methods) in 16 subjects. O = one determination in different individuals. ●, ■, □, = one subject with biopsies at different body weights.

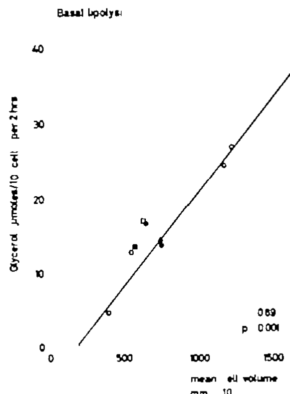
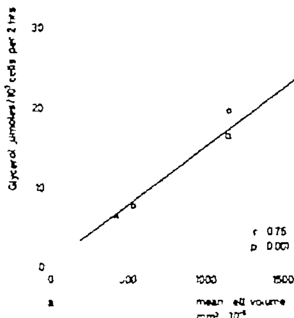
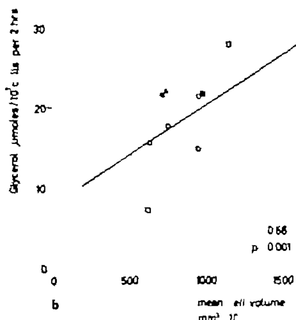


Fig. 2. Release of glycerol (μmoles) in vitro from 10^7 fat cells during two hours from adipose tissues with different mean fat cell volumes. The incubation medium consisted of 3 ml of Krebs-Henseleit bicarbonate buffer containing 3% bovine serum albumin and 100 mg glucose 100 ml.





fat cells ranged from 200 to 1 584 $mm^3 \times 10^{-6}$ and the average cell diameters from 68 to 142 μ. The loss of body weight after the intestinal bypass operation resulted in a pronounced decrease in the size of the mean cell volume of all six patients (Table II). In four of these six patients the changes followed closely the slope of the regression equation calculated for the mean cell volume versus the relative body weight of the donors in all 29 experiments (Fig. 1).

The release of glycerol into the bicarbonate buffer containing only albumin and glucose (1 mg/ml), was designated the basal lipolysis. This was significantly correlated with the mean volume of fat cells ($r = 89$ $p < 0.001$) (Fig. 2). 10^7 fat cells with mean volume 1 500 $mm^3 \times 10^{-6}$ released glycerol at a rate of 32 μmoles/2 hours, which was more than four times as rapid as for small fat cells (500 $mm^3 \times 10^{-6}$). Furthermore it was observed that the decrease of cell volume in one and the same individual (intestinal shunt operated) resulted in a diminished rate of glycerol release. These changes were related to the regression line common to all experiments.

An increased rate of lipolysis above the basal level was obtained with all agents added to the incubation medium. This stimulation of the lipolysis was significantly correlated with the mean volume of the fat cell ($p < 0.001$) (Table III). The increments in the glycerol production evoked by noradrenaline and dibutyryl cAMP are illustrated in Fig. 3.

From the regression lines it appears that the small fat cells responded less markedly to noradrenaline and isopropylnoradrenaline than to dibutyryl cAMP and theophylline. These findings were more apparent when the lipolytic response to the agents is expressed as the ratio between the stimulated glycerol release (Δ) and the basal glycerol release (relative lipolytic response). The noradrenaline and the dibutyryl cAMP experiments are presented in Fig. 4. If so calculated, the lipolytic response to dibutyryl cAMP ($r = -0.44$ $p < 0.05$) was negatively correlated to the mean volume of the fat cell. No correlation,

Fig. 3. Effect of noradrenaline (*a*) ($2 \cdot 10^{-6}$ M) and dibutyryl cAMP (*b*) (10^{-4} M) on the release of glycerol from 10^7 subcutaneous fat cells, calculated as the delta values above the basal glycerol production. Incubation conditions and symbols as in Fig. 2.

Table III. *Correlation coefficient and regression equation for the relations between the glycerol release stimulated with different agents and the mean volume of the subcutaneous fat cells obtained from 16 individuals on one or several occasions (Tables I and II), altogether 29 experiments (n = 29)*

	Stimulated lipolysis		Stimulated lipolysis/basal lipolysis	
Additions		Regression equation		Regression equation
Noradrenaline (2×10^{-6} M)	+0.75 $p<0.001$	$Y=0.014X+0.40$	−0.20 $p<0.30$	$Y=-0.0002X+1.01$
Isopropylnoradrenaline (2×10^{-8} M)	+0.67 $p<0.001$	$Y=0.023X-5.16$	+0.14 $p<0.50$	$Y=0.0002X+0.69$
Dibutyryl cAMP (10^{-3} M)	+0.66 $p<0.001$	$Y=0.013X+7.73$	−0.44 $p<0.02$	$Y=-0.0015X+2.66$
Theophylline (10^{-3} M)	+0.68 $p<0.001$	$Y=0.011X+3.80$	−0.44 $p<0.05$	$Y=-0.0052X-1.31$
Noradrenaline + (2×10^{-6} M)	+0.78 $p<0.001$	$Y=0.016X+4.59$		
Phentolamine HCl (0.05 µg/ml)				

on the other hand, existed between the lipolytic responses to either noradrenaline or isopropylnoradrenaline versus the mean cell volume.

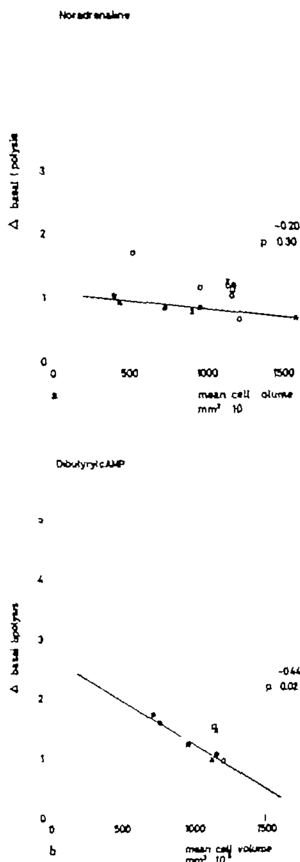
It is obvious from Table III that the regression lines for noradrenaline and for noradrenaline plus phentolamine run almost parallel ($b=0.14$ and 0.16, respectively), whereas the slope for isopropylnoradrenaline was somewhat steeper ($b=0.23$). Since in each experiment the smaller concentration (0.5 µg/ml) of phentolamine resulted in a more pronounced stimulation of the noradrenaline-induced lipolysis than did the higher phentolamine concentration, only the former data are charted here.

DISCUSSION

In this study the larger fat cells (1 500 $mm^3\times10^{-6}$) released glycerol under basal conditions at least four times as rapidly as did fat cells which had a mean volume of 500 $mm^3\times10^{-6}$. This would indicate that the basal lipolysis is not strictly surface-dependent, since the surface area of large cells is less than four times, or $\sqrt[3]{3^2}$ as large as that of the smaller cells. As a matter of fact the basal lipolysis seems to be either solely volume-dependent or even more rapid from large cells than could be expected from the larger volume. A possible explanation would then be that large fat cells release glycerol more rapidly than small fat cells because their uptake of glucose occurs faster (4, 6, 35). This would result in enhanced liberation of glycerol by two different mechanisms, either because of increased esterification of FFA, with subsequent reduction in the tissue FFA (3, 8), or because of direct stimulation of the lipase system (11, 16).

All agents used, noradrenaline, isopropylnoradrenaline, theophylline, and dibutyryl cAMP increased the rate of lipolysis. The stimulation above the basal glycerol release was well correlated with the size of the fat cells. Although each agent was used in a concentration known from a considerable number of studies to induce maximal lipolysis, certain differences were observed between the four regression curves (Table III). Assuming that the diffusion of any agent is not the rate-limiting factor, this difference in the curves could be attributed to the different sites of action of the four agents and to different metabolic characteristics of small and large fat cells.

As regards the effects of noradrenaline and isopropylnoradrenaline, no significant correlation was observed between the relative lipolytic response to these agents and the mean cell volume. 10^7 cells of large-sized cells (1 500 $mm^3\times10^{-6}$) increased their rate of glycerol release by 22 µmoles/2 hours when exposed to noradrenaline. The corresponding value for the small fat cells (500 $mm^3\times10^{-6}$) was about 7 µmoles. In other words the lipolytic response to noradrenaline,



calculated per cell number seems to be independent of the cell size.

Recently Hartman et al. (17) have made similar observations with noradrenaline on lipolysis by isolated fat cells of the rat epididymis. These authors concluded that the number of adrenergic receptors is fixed in the fat cells. The findings (7 27 28 29) in human adipose tissue that noradrenaline exhibits both α- and β-adrenergic properties, with inhibitory and stimulatory effects on lipolysis, respectively motivate at least one modification of this suggestion, namely that it is the net balance between the α and β-adrenergic effects which is fixed in the human fat cell. We have observed that phentolamine induced almost the same, absolute increase in the lipolysis by small and large cells. Taken together with the observation of the steeper slope of the regression line for isopropylnoradrenaline than for noradrenaline, this indicated that the α-agonistic effect of noradrenaline is relatively less pronounced for large fat cells. The value of this finding is diminished by the fact that only two concentrations of phentolamine were used. In the present study we have only some indirect evidence of diminished maximal lipolysis by increasing cell volume. Thus dibutyryl cAMP stimulated the lipolysis of large fat cells by less than 50% of the basal lipolysis (Δ = 28 μmoles/10^7 cells/2 hours) whereas the lipolysis by small adipocytes was stimulated by 230% (Δ = 14 μmoles/10^7 cells/2 hours). In other words, when subcutaneous fat cells of equal weight are compared, the tissue composed of small cells is the most sensitive to dibutyryl cAMP. A similar conclusion can be drawn from studies undertaken with theophylline. In general, the lipolysis induced with theophylline is somewhat less than that induced with dibutyryl cAMP. This would be explained best by the fact that the phosphodiesterase activity is not completely blocked by theophylline even at high concentration.

In part, the present studies are in consonance with the results of Zinder and Shapiro (37), who studied the effect of adrenaline and ACTH on

Fig. 4. The stimulatory effect, i.e. the relative change in comparison with the basal lipolysis, of noradrenaline (*a*) (2 10^{-6} M) and dibutyryl cAMP (*b*) (10^{-3} M) on the release of glycerol from 10^7 subcutaneous fat cells. Incubation conditions and symbols as in Fig. 2.

the release of FFA *from* isolated *fat* cells of the rat epididymis. Thus we have observed that the lipolysis per cell number is positively correlated with the cell size. Unlike these authors, we have seen no negative correlation between the catecholamine-induced glycerol release per weight unit of tissue and the cell surface. On the other hand dibutyryl cAMP and theophylline induced in human fat cells a release of glycerol per weight of tissue which was negatively correlated with the cell size. In other words the small fat cells were less sensitive to noradrenaline and isopropylnoradrenaline than to dibutyryl cAMP and theophylline, whereas no such difference was found for large fat cells. If these findings in vitro have any relevance for the metabolic situation in vivo, it would mean that small fat cells increase their glycerol production more rapidly under conditions of maximal lipolysis, i.e. insulin-deficient states. Adipose tissue of large cells would then respond with less marked lipid mobilization than adipose tissue composed of small cells. This could be *one contributing factor to* the absence of ketosis in aged people with severe hyperglycemia and even coma (1 23).

ACKNOWLEDGEMENTS

The investigation was supported by grants from the Ernst Nathorst Foundation and the Swedish Medical Research Council (B72 19x 1034).

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Congress Announcement

The Second World Congress on Ultrasonics in Medicine will be held in De Doelen Congress Centre in Rotterdam, The Netherlands, June 4–8, 1973 under the auspices of the Medical Faculty of the Erasmus University in Rotterdam and the World Federation for Ultrasound in Medicine and Biology

Chairman. Dr M. de Vlieger lecturer in neurology of the Erasmus University

Main themes. Current developments in ultrasonics in physics, neurology internal medicine, cardiology gynaecology and ophthalmology

Secretariat c/o Holland Organizing Centre, Lange Voorhout 16, The Hague, The Netherlands.

Acta med. scand. Vol. 193 pp. 477–480, 1973

TREATMENT OF PROGRESSIVE SYSTEMIC SCLEROSIS (PSS) WITH PENICILLAMINE

Preliminary Report of Two Cases

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Abstract. Longitudinal studies over two years of penicillamine treatment in two pilot patients with progressive systemic sclerosis are described. Improvement was seen in both cases, though not in all later patients. It would appear that small doses, as compared to those in Wilson's disease, over long periods are the appropriate scheme for penicillamine therapy in this disease. The effective dosage appeared to be very variable. Desensitization should be tried when side-effects occur. There are indications that the soluble/insoluble collagen ratio in the dermis may generally serve as an overall rough objective parameter for clinical improvement.

From their studies on dermal collagen in various disorders and its response to penicillamine, Harris and Sjoerdsma (10) suggested the use of penicillamine for the treatment of scleroderma in 1966. As shown by Nimni and Bavetta (16) D-penicillamine (β,β-dimethylcysteine) increases the percentage of soluble dermal collagen and decreases its intramolecular cross-linking. Furthermore Uitto et al. (18) demonstrated that penicillamine reduced both the biosynthesis of hydroxyproline and the conversion of soluble into insoluble collagen in scleroderma.

Although fully conscious of the reported side-effects of penicillamine, the above mentioned reports and the fact that penicillamine has been used in Wilson's disease since 1956 (19) made trials with this drug appear justified in PSS, for which no universally accepted treatment yet exists. Success (5), failure (9) and reserve (4, 20) in clinical studies of penicillamine in PSS have been reported.

In our initial longitudinal studies of two PSS patients we tried to find out whether penicillamine is indeed beneficial in PSS and whether the soluble/insoluble collagen ratio could serve as an objective parameter for clinical improvement.

METHODS

A modified technique for determination of the collagen ratio, derived from various described methods (1, 2, 7, 12), was first tried out on skin specimen from an operated breast resulting in an S.D. of about 10%. For single tests of the very small biopsies from the affected skin of PSS patients, however, the S.D. proved to be about 20% in normal skin specimen.

Clinical parameters consisted of: (*a*) pliability of the affected skin, (*b*) measurement of maximum spontaneous finger joint flexions with a special instrument consisting of two metal semicircles with joint central axis, and (*c*) follow-up roentgencinematography (8) of the digestive tract.

CASE HISTORIES

Case 1

Woman, born 11.1.1915. From 1916 onwards, starting with Raynaud's syndrome of the fingers, PSS gradually developed notwithstanding bilateral cervical sympathectomy in 1947. In 1952 amputation of necrotic fingertips following delivery of youngest child and use of uterotonics. Over the years many advocated treatments for PSS were tried without lasting success, except for prednisone, which had to be discontinued when a gastric ulcer developed.

Penicillamine treatment was started in Feb. 1969 with 250 mg, over the next days increasing as follows: 500–500–750–750–750–1 000–1 500–1 500–1 500 mg (10th day), aimed at obtaining a similar dosage to that used in Wilson's disease. On the 10th day however, the patient developed fever, pain in the nape of the neck, and an urticarial rash. The temperature remained above 39°C for 3 days and at about 38°C for 11 consecutive

Fig. 1. Case 1. Graphical presentation of penicillamine dosage, mean maximum metacarpophalangeal flexion, and soluble/insoluble collagen ratio in successive biopsies of the affected skin from one area.

Apart from the one marked increase of soluble/insoluble collagen ratio, there is no correlation between clinical improvement and biochemical findings.

(The initial soluble/insoluble collagen ratio of the biopsy of the skin of an affected area in this patient, however was higher than the similar ratio found in the apparently normal skin of the operated breast.)

... Histology of the rash (Dr J. Spaas): picture compatible with drug allergy. At the end of March penicillamine was readministered by increasing daily doses in mg: 0-50-50-100-100-100-100-250-250 (10th day), when again she developed fever (39°C) for 1 day and painful joints, but no rash. In mid-April readministration of penicillamine: 100 mg for 19 days, 2 × 100 mg for 10 days, followed by a maintenance dose of 3 × 100 mg/day for 3 years without reappearance of side-effects.

Clinical improvement was evident but appeared to be not clearly correlated with increase of the soluble/insoluble dermal collagen ratio (Fig. 1). Remarkably both reactions on penicillamine appeared on the 10th day and clinical improvement, even during the winter was obtained with a low dosage.

Case 2

Young girl, born 16.3.1949. From the age of 12 she gradually developed PSS. Penicillamine treatment was started in mid-February 1969 with gradually increasing doses up to 1 400 mg (after 9 days) the maximum dose tolerated by her stomach, and continued for over 2 years. In the 3rd year a leukopenia forced us to discontinue the therapy. The distinct improvement in our three clinical parameters appeared to be associated with an increase of the soluble/insoluble collagen ratio (Fig. 2).

The success in these two patients prompted us to treat other patients (9 with PSS and 2 with localized scleroderma) with penicillamine, generally in low doses as compared to those in Wilson's disease.

DISCUSSION

Since the half life of the insoluble fraction of collagen (13) is between 100 days and 1 year depending on the age of the individual, we consider that 2 years would be a reasonable time for follow-up treatment to permit adequate evaluation. From this pilot study it would appear that, if penicillamine is tolerated, it may be useful in some cases of PSS.

In the first patient penicillamine (as spontaneously reported) apparently favourably influenced the frequency and severity of spastic pains in the fingers only after 2 years, with even pain-free days during wintertime. Increasing small doses of penicillamine, up to about 300 mg/day for a prolonged period may reduce side-effects and appear to be the ideal therapeutic scheme for PSS. This is in agreement with the suggestion of Bluestone

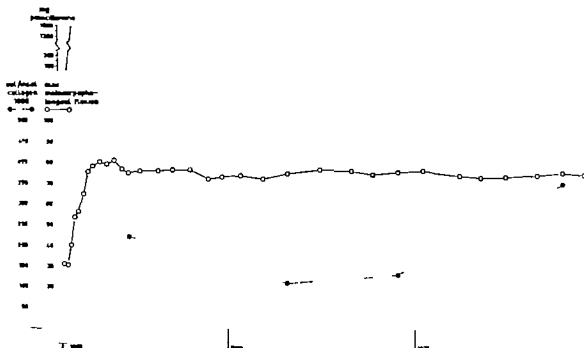


Fig. 2. Case 2. Similar graphical presentation as in Fig. 1. There appears to exist some correlation, but fluctuative, between the soluble/insoluble collagen ratio and mean maximum flexion of the finger joints.

et al. (4). This scheme has so far resulted in only 2 drop-outs in a total of 11 patients as compared to 8 out of 11 of the patients of Bluestone et al. Our initial objective to treat with similar dosages as in Wilson's disease appeared to be inappropriate. Desensitization (14–17) must be considered if side-effects occur as described in case 1.

A certain correlation between clinical improvement and biochemical soluble/insoluble collagen ratio was observed in case 2 only, yet with obvious fluctuations. In the literature at our disposal we could not find longitudinal studies of this ratio in human skin during various seasons. Furthermore, in one case a controversial response in the various affected areas of the skin was observed, i.e. improvement of most sclerotic parts and hardening of other affected areas of the skin. In this connection we would like to mention that Bluestone et al. (4) also found bizarre curves in their studies of skin elasticity in vivo.

We tend to agree with the suggestion of Keiser and Sjoerdsma (11) that earlier treatment might improve the results, as an additional fresh case responded dramatically within 10 days. In shorter follow-up of additional patients with various types of scleroderma treated with penicillamine, similar results were obtained in those cases which appeared to respond to the treatment.

The question arises whether the administration of penicillamine has to be continued forever or can be discontinued, and if so when. The observations of Bluestone et al. seem to indicate that the treatment should not be discontinued, which is feasible since penicillamine has been used in some cases of Wilson's disease from 1956 onwards (19). On the other hand, we must also bear in mind that PSS may clinically burn out with age. It would therefore appear that only the patient's clinical course will serve as guideline whether to prolong or discontinue treatment. However, it is advisable that penicillamine had better be discontinued during pregnancy, although the observation of Mjølnerød et al. (15) seems to be exceptional.

The poor chance of collecting sufficiently large numbers of patients, the partial efficacy of penicillamine in PSS, and the insufficient reliability of the soluble/insoluble collagen ratio as an objective parameter make it next to impossible to carry out, respectively, double-blind and double-

blind cross-over trials to assess the beneficial effects of penicillamine in PSS beyond doubt.

Further studies with technical clinical parameters which can be expressed numerically such as measurement of pressure and electromyography of the oesophagus and of lung function tests, are in progress.

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Acta med. scand. Vol. 193 pp. 481–482, 1973

EDITORIAL

CLINICAL EVALUATION OF ANTIARRHYTHMIC DRUGS

The number of drugs on the market is steadily increasing even though a definite slowing down has been noticed after the thalidomide tragedy. The importance of reliable methods for clinical evaluation of drugs is apparent. The question is not only to prove that a certain drug has few and innocent side-effects but also to show by controlled studies that it is effective. These problems are especially difficult in patients with predominantly subjective symptoms such as angina pectoris. The introduction of the β-blocking agents was followed by a definite improvement in the methodology of evaluation of drugs against angina pectoris (3).

Unfortunately this methodological improvement has not penetrated all other fields of clinical research, and the need for randomized, clinical drug trials in certain diseases, including malignant states, was recently discussed (1). A review of the literature reveals that clinical evaluation of antiarrhythmic drugs is without doubt a field in which a methodological improvement could, and should, come. There are several reasons. One is the great spontaneous variation in the frequency of arrhythmic episodes documented by, for example, a continuous ECG recording with the aid of a portable tape recorder. Furthermore the mode of reporting subjective experience of the arrhythmias differs much between patients.

From this point of view the arrhythmias can be divided into three groups: 1) attacks of paroxysmal tachycardia, 2) continuous chronic states with single or multiple premature beats, 3) arrhythmias in connection with an acute disease such as myocardial infarction.

1) Bouts of paroxysmal tachycardia are the only type of arrhythmia in which I think it is possible to rely solely on the patient's history and this is so only if the patient carefully notes the time of start and end of each single attack. Great difficulties may appear when a large proportion of these tachycardias start during sleep.

Furthermore it is necessary to observe the patient over a long period, both when on the active drug and when on placebo. The spontaneous variation of these paroxysmal tachycardias is great and dependent, among other things, on changes in the condition of the gastrointestinal tract.

2) Continuous chronic arrhythmia consisting of single or multiple premature beats is not uncommon and is sometimes a benign state, but arrhythmias of this type have also been found in a high percentage in patients who later develop life-threatening arrhythmias. A detailed study of such patients reveals a pronounced variability in the number of premature beats. With the aid of a portable tape recorder (2) ECG was recorded around the clock. To get a representative sample but to reduce the amount of information, the first 10 min of each hour were transferred to an ECG machine and the written ECG was analysed in detail as regards frequency of premature beats per minute. A surprising variation in the frequency could be seen. In one patient no premature beats were recorded during the first 5 min of such a 10-min period, while during the last minute of the same 10-min period 56 premature beats of one type and 5 of another were found. It is easy to understand how diverging results will be obtained for a drug in such a patient, depending merely on the time of injection. If the "right" moment is chosen in a short-term experiment, the same drug may be either an excellent arrhythmia-producing agent or a good antiarrhythmic compound!

Similar great variations in the frequency of premature beats were found when the means of all the 24 10-min periods were calculated.

For this reason a model has been constructed for evaluation of antiarrhythmic drugs in patients

with premature beats of this type, during an initial period of 2–3 days the patient carries his tape recorder to become acquainted with it and no drug is given. After this initial period, which is not necessary in all patients, the drug is given in increasing dosage with daily recording of side effects. Preliminary results regarding the arrhythmia are checked with an oscilloscope during some minutes. In this way the optimal dosage of the drug is obtained and given the patient during a week. Afterwards placebo tablets are given also during a week after an intermission of 3 days to avoid a possible remaining effect of the active drug. ECG is continuously recorded throughout this period and afterwards analysed according to the above mentioned schedule.

3) Evaluation of a new drug in arrhythmias in connection with an acute disease such as a myocardial infarction offers specific problems. Ethical aspects become dominant, since it is not possible to continue with a new and comparatively unknown drug if a serious arrhythmia appears. In such a case it will be imperative for ethical reasons to switch over to a generally accepted drug—but this will also mean a reduction of the observation period for the new drug and the essence of the controlled study disappears, with concomitant difficulties in evaluation of the new compound.

Also in these patients with an acute infarction the frequency of the arrhythmias shows great variations. Therefore a control material is necessary also in this type of studies. The controls can be selected in different ways, most easily perhaps by using the date of birth—patients being born on an even date are selected for the drug and patients born on an uneven date for the control (which might be a placebo or a well established drug). It is important that the general treatment, including analgetics and i.v. infusions, is the same in the two groups.

The evaluation of the antiarrhythmic effect of a drug in such patients has recently been described by Mogensen (4), who used continuous ECG recordings in these patients and calculated the frequency of the different arrhythmias, the ventricular premature beats were also classified as monofocal, multifocal, paired, R on T. All R-R intervals exceeding 3 sec were noted, as well as the occurrence of A V block and episodes of ventricular tachycardia.

Studies of the type scheduled above are not frequent in the literature. In fact they are almost non-existent. The reason is obvious: studies of this type are time-consuming and cumbersome. Nevertheless they are necessary to get a true evaluation of the effectiveness of antiarrhythmic drugs. In these days of abundance of scientific papers it might be appropriate to quote William Withering in his treatise on foxglove: "It is now about ten years since I first began to use this medicine. Experience and cautious attention gradually taught me how to use it. For the last two years I have not had occasion to alter the modes of management, but I am still far from thinking them perfect"

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Acta med. scand. Vol. 193 pp. 483–491 1973

CYTOSTATIC TREATMENT OF GLOMERULAR DISEASES

I. *Effect of Azathioprine on Serum Creatinine and Proteinuria*

Report from a Copenhagen Study Group of Renal Diseases

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Abstract. Forty-six patients with chronic glomerular diseases have been treated with azathioprine for up to 84 months. The importance of the variables sex, age, clinical diagnosis, histological glomerular changes, arterial hypertension, treatment with prednisone and initial values of serum creatinine and proteinuria was investigated. In 22 patients the renal state as estimated by serum creatinine, proteinuria and urinary sediment was normalized or improved. The renal state was unchanged in 9 patients and deteriorated in 12. The clinical diagnoses glomerulonephritis and idiopathic nephrotic syndrome, normal arterial BP and normal initial serum creatinine concentration were correlated to the improvement. Five of 26 patients deteriorated after withdrawal of azathioprine. Eighteen patients died during the study: 7 from uremia, 2 from side-effects of the cytostatic treatment and 9 from other diseases. Initial serum creatinine concentration was the only variable correlated to survival.

Several studies on the effect of cytostatic treatment on different types of renal diseases have been published in recent years. Favourable results have been reported from controlled studies in idiopathic nephrotic syndrome in children (2). In other types of renal diseases controlled studies have failed to show any remarkable improvement from treatment with cytostatic drugs alone or in combination with glucocorticoids (4, 7, 9). Michael et al. (8) treated 28 patients with progressive life-threatening renal diseases with a combination of azathioprine and prednisone. In 70% of the patients they observed an improvement which was ascribed to the treatment.

The present study represents an analysis of serum creatinine concentration, proteinuria, urinary sediment and survival rate in 46 patients with glomerular diseases during treatment with azathioprine for up to 84 months and after withdrawal of the treatment (26 patients). Furthermore, the importance of the variables sex, age, clinical diagnosis, histological glomerular changes, arterial hypertension, treatment with prednisone and initial values of serum creatinine concentration and proteinuria was investigated.

MATERIAL AND METHODS

Admission of patients to the trial was based on the following criteria.

1. Histological glomerular changes. 2. At least one of the following signs: (*a*) decreased glomerular filtration rate as estimated by means of creatinine clearance, (*b*) proteinuria (> 200 mg/24 h), (*c*) pathological urinary sediment on microscopy. 3. Lack of remission. 4. No previous treatment with cytostatic drugs.

In view of the lack of remission before treatment the patients were used as their own controls. The average duration of the renal disease before start of the cytostatic treatment was two years.

The trial did not include renal diseases due to diabetes mellitus, amyloidosis, multiple myeloma, renal vein thrombosis, arterial hypertension, pyelonephritis, toxic nephropathy secondary syphilis or constrictive pericarditis.

Twenty-four of the 46 patients were females and 22 males (4. adults and 4 children). The average age was 35 years (range 5–72).

The clinical and histological diagnoses appear from

with premature beats of this type: during an initial period of 2–3 days the patient carries his tape recorder to become acquainted with it and no drug is given. After this initial period, which is not necessary in all patients, the drug is given in increasing dosage with daily recording of side-effects. Preliminary results regarding the arrhythmia are checked with an oscilloscope during some minutes. In this way the optimal dosage of the drug is obtained and given the patient during a week. Afterwards placebo tablets are given also during a week after an intermission of 3 days to a old a possible remaining effect of the active drug. ECG is continuously recorded throughout this period and afterwards analysed according to the above mentioned schedule.

3) Evaluation of a new drug in arrhythmias in connection with an acute disease such as a myocardial infarction offers specific problems. Ethical aspects become dominant, since it is not possible to continue with a new and comparatively unknown drug if a serious arrhythmia appears. In such a case it will be imperative for ethical reasons to switch over to a generally accepted drug—but this will also mean a reduction of the observation period for the new drug and the essence of the controlled study disappears, with concomitant difficulties in e aluation of the new compound.

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4. Lobular glomerulopathy

Mesangial cell proliferation with accumulation of basement membrane-like material in the mesangial regions forming centrolobular nodules with margination of the capillaries. Thickening of the capillary wall accompanied by double basement membrane can be seen in PASM-stained thin section.

5. Extracapillary glomerulopathy

Generalized mesangial and epithelial proliferation in the tuft accompanied by large number of epithelial crescents in the capsule (>20%). In the most severe cases (corresponding to rapidly progressive nephritis) the crescents involve most or all corpuscles and may occupy the whole capsular circumference, compressing the tuft.

6. Membranoproliferative glomerulopathy

Proliferative changes (mainly mesangial) accompanied by thickening of the capillary wall. Both changes are generally diffuse. In PASM-stained thin sections double basement membrane may be seen, but occasionally spikes may be found in some segments.

7. Epimembranous glomerulopathy

Diffuse and generalized thickening of the capillary walls. In PASM-stained thin sections the characteristic "spiky" projections on the outer (subepithelial) aspect of the basement membrane can be seen. The capillary wall may be greatly thickened when the spikes gradually encircle the deposits, forming a "tornade". Proliferative changes are absent.

8. Segmental-focal glomerulosclerosis

The glomerular sclerosis is found only in some glomeruli, and in some segments only while the remaining parts of the tuft show no or only minimal changes. The sclerosed areas are generally hypocellular and may be adherent to the capsule. (This type was not represented in the study.)

9. Unclassifiable lesions

(*a*) Mixed, atypical forms. (*b*) End stage kidneys.

Laboratory Investigations

The glomerular filtration rate was estimated by means of creatinine clearance or serum creatinine, which were determined at 2-week intervals. At similar intervals examination of urinary sediment, quantitative determination of urinary proteins, and arterial BP measurement were performed. For all patients the quantitative determinations of urinary protein excretion were carried out in single laboratory (K.H.) on 24-hour specimens. The method used involved precipitation with Tsuchia's reagent, isolation by centrifugation and washing of the precipitate, which was then dissolved in sodium hydroxide. The protein content was determined photometrically after addition of biuret reagent. During the treatment the following blood analyses were controlled at 1- or 2-week intervals: Hb, leucocytes, platelets, reticulocytes and ESR. Furthermore, the concentrations of albumin, α_2-macroglobulin, IgG, IgA, IgM and transferrin in serum as well as in the urine were examined. In some of the patients the deposits of immunoglobulins in the renal biopsy were estimated. In addition humoral and cellular hypersensitivity were estimated after vaccination with different types of antigens.

The present report includes only the analyses of serum creatinine, proteinuria and urinary sediment performed at 2-month intervals.

Treatment

Patients with idiopathic nephrotic syndrome were not admitted to the trial unless treatment with glucocorticoids (prednisone 0.65 mg/kg b.wt./24 h for at least months) had proved unsuccessful, involved serious side-effects, or had been necessary for more than one year.

The remaining patients were treated with azathioprine (Imurel® supplied by Burroughs Wellcome & Co) in doses of 4 mg/kg b.wt. initially decreasing to 1.5 mg kg b.wt. after 6 weeks. The treatment was combined with glucocorticoids in case of severe progression. In 17 patients only cytostatic treatment pplied. In the remaining 29 patients azathioprine was combined with prednisone for variable period.

Assessment

The patients were evaluated at two main stages of the treatment.

(*a*) After treatment for up to 12 months. This study included the periods 12 months, the first 4 months, the periods from start to the latest recorded value up to 12 months and the period from the fourth month up to the latest recorded value.

(*b*) After treatment for up to 84 months. This study included the period from start to the latest recorded alue up to 84 months as well as the period from withdrawal of the cytostatic treatment to the final evaluation.

The renal state was classified as *normalized*, *improved*, *unchanged*, or *deteriorated* according to the following criteria. *Normalized*: serum creatinine concentration <1.5 mg% proteinuria <200 mg/24 h and normal urinary sediment on microscopy. *Improved*: decrease in proteinuria of 50% of the initial value and/or decrease in serum creatinine concentration of 25% of the initial alue, alternatively change from pathological to normal value. *Deteriorated*: an increase in proteinuria of 50% and/or an increase in serum creatinine concentration of 25% of the initial value, alternatively change from normal to pathological value. Deterioration of one of the variables was considered deterioration of the renal state regardless of an improvement in the other variables. *Unchanged*: changes within the limits given above.

Statistical Analysis

The object of the statistical analyses in the present study has been search of reasonable structures in the data on the basis of limited amount of advance information rather than a test of hypotheses. Therefore the data ere analysed in all combinations of variables. The reliability of the results must be evaluated against this background.

It has been difficult to make presumptions about distributions, because the 46 patients make up homogeneous

Table III. *Serum creatinine concentration during treatment with azathioprine up to 12 months*

F = Friedman test, based on available observations every second month. W = Wilcoxon signed-rank test, based on the difference between first and last observation in % of the mean of these two observations. *N* = number of patients, *p* = statistical results ≤5% level of probability. ↓ = statistically significant decrease, ↑ = statistically significant increase, } = statistically significant difference between the groups embraced by the bracket (Wilcoxon, Spearman rank or Kruskal-Wallis tests)

	Change from											
	Start to 4th mo. F		Start to 12th mo. F		Start to 4th mo. W		Start to 12th mo. W		Start to latest value W		4th mo. to latest value W	
Grouping	*N*	*p*	*N*	*p*	*N*	*p*	*N*	*p*	*N*	*p*	*N*	*p*
No grouping	39		22		39		24		46		35	
Sex ♂	17		11		17		12		22		16	
♀	22		11		22		12		24		19	
Age (y) 0–19	8		7		8	↓	7	}	9		7	
20–49	21		10		21		12	}	24		20	
≥50	10		5		10		5	}	13		8	
Clinical diagnosis												
Connecth. tissue diseases	11		7		11	↓	8		15		10	↑ }
Glomerulonephritis	19		9		19		10		22		16	}
Idiopath. nephrotic syndr.	9		6		9		6		9		9	}
Prednisone at start												
Given	31		13		20		14		4		18	
Not given	8		9		19		10		22		17	
Prednisone during study												
Given	31		19		31		20		37		28	
Not given	8		3		8		4		9		7	
Hypertension												
Present	9		3		9		4		11		8	
Not present	30		19		30		20		35		27	
creatinine at start												
≤1.5 mg/100 ml	24		18		24	}	18		29		23	
>1.5 mg/100 ml	15	↓	4		15	↓ }	6		17		12	
Proteinuria at start												
≤2 g/24 h	8	↓	4		8		4		9		6	
>2 g/24 h	31		18		31		20		37		29	
Glomerular lesions												
Minimal changes	8		5		8		5		8	}	8	↓ }
Proliferative	8		4		8		5		10	↑ }	8	↑ }
Extracapillary	9	↓	4		9	↓	4		10	}	7	}
Others	14		9		14		10		18	}	12	}
Deaths during study	6		1		6		1		9		4	
Alive during study	33		21		33		23		37		31	
Deaths from uraemia	2		0		2	}	0		4		1	
Alive or death from other causes	37		22		37	}	24		42		34	

group only in a few aspects. Therefore only non-parametric methods were used (10). Wilcoxon signed-rank test, Friedman test, Wilcoxon Mann-Whitney test, Kruskal-Wallis test, Spearman rank-correlation with *t*-test and χ^2-test.

For the analysis of developments the Friedman test was used, based on the hypothesis that there were only random variations between the 2-month observations, and the alternative hypothesis that an unknown development was common to the patients.

RESULTS

The initial values of serum creatinine in patients with connective tissue diseases and glomerulonephritis were higher than those of the patients

Table IV *Proteinuria during treatment with azathioprine up to 12 months*

Abbreviations and symbols as in Table III

	Change from											
	Start to 4th mo. F		Start to 12th mo. F		Start to 4th mo. W		Start to 12th mo. W		Start to latest value W		4th mo. to latest value W	
Grouping	*N*	*p*	*N*	*p*	*N*	*p*	*N*	*p*	*N*	*p*	*N*	*p*
No grouping	38		22	↓	38	↓	24	↓	45	↓	33	↓
Sex ♂	17		11	↓	17	↓	12	↓	21	↓	16	
♀	21		11	↓	21		12	↓	24	↓	17	↓
Age (y.) 0–19	8		7	↓	8		7	}	9	↓	8	}
20–49	21		11		21		13	}	23		21	}
> 50	9		4	↓	9		4	}	13	↓	5	}
Clinical diagnosis												
Connective tissue diseases	10		6		10		7		14		9	
Glomerulonephritis	19		10	↓	19		11	↓	22	↓	15	↓
Idiopath. nephrotic syndr.	9	↓	6	↓	9		6	↓	9	↓	9	
Prednisone at start												
Given	20		14	↓	20	↓	15	↓	23	↓	19	
Not given	18		8	↓	18		9		22		14	
Prednisone during study												
Given	30		19	↓	30	↓	20	↓	36	↓	27	
Not given	8		3	↓	8		4		9		6	
Hypertension												
Present	9		3		9		4		11		8	
Not present	29		19	↓	29		20	↓	34	↓	25	↓
Serum creatinine at start												
< 1.5 mg/100 ml	24		17	↓	24		19	↓	29		24	↓
> 1.5 mg/100 ml	14		5		14		5		16		9	
Proteinuria at start												
< 2 g/24 h	8		4		8		4		9		6	
> 2 g/24 h	30		18	↓	30	↓	20	↓	36	↓	27	↓
Glomerular lesions												
Minimal changes	8		5	↓	8		5		8	↓	8	
Proliferative	8		4		8		5		9		7	
Extracapillary	8		3		8		3		10	↓	5	
Others	14		10		14		11		18		13	
Deaths during study	6	↓	1		6	}	1		8	}	3	
Alive during study	32	↓	21	↓	32	↓ }	23	↓	37	↓ }	30	↓
Deaths from uraemia	2		0		2		0		3	}	0	
Alive or death from other causes	36		22	↓	36	↓	24	↓	42	↓ }	33	↓

with idiopathic nephrotic syndrome, which were all within the normal range (Table I). By contrast, the initial proteinuria in the three clinical groups did not differ

Analysis of initial serum creatinine concentrations in the seven histological groups in the study showed lower values in the two groups "minimal changes" and "epimembranous glomerulopathy" when compared to the five other groups (Table II). Again no differences could be demonstrated between the initial proteinuria of the seven groups.

When all patients were considered as one group, no changes were found in the *serum creatinine* concentrations during the observation period (Table III).

Subdivision of the patients according to the variables given in Table III indicated the im-

Table V *Summary of renal state in 46 patients after treatment with azathioprine for up to 12 and 84 months*

Renal state[a]	No. of pats.	Duration of treatment (mo.) Median	Range
After 12 mo.			
Normalized	5	12	12 12
Improved	19	12	4–12
Unchanged	12	12	2–12
Deteriorated	10	6	2–9
After 84 mo.			
Normalized	8	42	18–84
Improved	14	18	4–60
Unchanged	9	13	2–41
Deteriorated	15	9	3–69

As estimated by serum creatinine and proteinuria.

portance of the variables age, clinical diagnosis, initial serum creatinine and proteinuria, and glomerular histology A decrease in serum creatinine concentrations was observed in patients below 20 years of age, patients with connective tissue diseases, patients with initial serum creatinine above 1.5 mg/100 ml and initial proteinuria below 2 g/24 h, as well as in patients with extracapillary glomerular lesions. The decrease could only be demonstrated after the first 4 months of but was not observed after 12 months treatment

Comparison of the different subgroups of the variables revealed only a few differences between the individual subgroups (Table III).

There was a statistically significant decrease in the *proteinuria* during the treatment (Table IV). In contrast to the changes in serum creatinine concentrations, the decrease in proteinuria extended beyond 4 months of treatment. The variables age, clinical diagnosis, arterial BP renal histology and initial values of serum creatinine and proteinuria seemed to be of importance. A decrease in proteinuria was observed in patients below 20 and above 50 years of age, patients with glomerulonephritis and idiopathic nephrotic syndrome, patients without arterial hypertension, patients with initial serum creatinine below 1.5 mg/100 ml and initial proteinuria above 2 g/24 h. Furthermore a decrease was observed in patients with minimal and extracapillary changes in the glomeruli and in patients who surviv ed during the observation period or died from causes other than uraemia. Possibly treatment with prednisone had some effect on the decrease in proteinuria, whereas no influence of sex could be demonstrated.

A statistical analysis was made of the relation between all the variables. No correlation was found between the changes in the serum creatinine concentrations and the proteinuria. The histological classification was correlated to the clinical diagnosis due to a correlation between "minimal changes" and idiopathic nephrotic syndrome, a consequence of the diagnostic criteria for this clinical diagnosis. Beyond this, no further information was obtained.

Classification of the patients according to renal state as normalized, improved, unchanged and deteriorated showed that about half of the patients were normalized and improved at the 12 month as well as at the 84-month stage (Table V). In one-fourth of the patients in the 12-month study and one-third of the patients in the 84-month study the renal state was deteriorated.

A statistical analysis was made of the correlation between the four subgroups of renal state and the variables sex, age, clinical diagnosis, prednisone treatment, arterial BP initial values of serum creatinine concentrations and proteinuria, and histological groups. At the 12-month examination there was a statistically significant shift of the number of patients towards normalized" in the two clinical subgroups glomerulonephritis and idiopathic nephrotic syndrome as compared to the connective tissue diseases. A similar trend was observed in patients without arterial hypertension, whereas no influence could be demonstrated by the other variables. At 84 months there was the same shift towards normalized renal state as described at 1 months. Furthermore, there was a shift towards normalized renal state in patients with initial serum creatinine concentrations below 1.5 mg/100 ml.

In 26 patients the azathioprine treatment was withdrawn because the disease was considered in a steady state without change after reduction of the dose of azathioprine. In five of these patients the renal state deteriorated after withdrawal (Table VI). In the remaining patients the renal state either remained unchanged or improved following withdrawal of azathioprine. A statistical analysis of the four subgroups of renal state against the other variables given above showed

a shift towards normalized state in the two clinical groups glomerulonephritis and idiopathic nephrotic syndrome.

Eighteen of the 46 patients died within 84 months from the start of azathioprine treatment (Table VII). Seven patients died from uraemia, two from side-effects of the treatment (pancytopenia and septicaemia) and nine from other causes, primarily cardiovascular diseases.

A statistical analysis of the group of patients who died during the study and the group of patients who survived, against the variables sex, age, clinical diagnosis, prednisone treatment, initial values of serum creatinine concentrations and proteinuria and histological classification showed a shift towards a greater number of survivals among patients with initial serum creatinine concentrations equal to or below 1.5 mg/100 ml as compared to those with serum creatinine concentrations higher than 1.5 mg/100 ml. No influence of other variables could be demonstrated.

An analysis of the two groups "death from uraemia" and "death from causes other than uraemia" against the variables given above revealed no influence of any of the variables.

The most common side-effects observed during the treatment with azathioprine were transitional dyspepsia (9 cases) and transitional bone marrow depression (anaemia 12 cases, leukopenia $< 2\,000/\mu l$ 9 cases, thrombocytopenia $< 50\,000/\mu l$ 6 cases). These side-effects were in particular seen at the start of the treatment. Fatal pancytopenia and septicaemia occurred in two patients. One patient had a severe anaphylactic reaction to azathioprine reflected in a sudden deterioration of the renal function (11).

DISCUSSION

In the present study the patients were used as their own controls. Consequently the results of the investigation must be considered in the light of the limitations implied in this type of clinical trial. This is of particular importance in the evaluation of the effect of the cytostatic treatment. The probability that the improvement observed during the study was caused by the treatment is, however increased by several factors. The observation period before start of the treatment was long. Thirty-seven of the 46 patients had definite structural glomerular changes, which are usually considered to carry a more serious prognosis than minimal or no glomerular changes. Finally deterioration was observed in some of the patients after withdrawal of the treatment.

The serum creatinine concentration showed only minor changes during the study. The decrease observed in a few of the subgroups after the initial four months of treatment could not be demonstrated beyond this period. On the other hand, the absence of an increase in the serum creatinine concentration may also reflect an effect of the cytostatic drug.

In the present study the most marked alteration was a decrease in the proteinuria observed in all patients considered as one group as well as in several subgroups of patients. The decrease in proteinuria could not be explained by a simultaneous decrease of the glomerular filtration rate, as no correlation was found between the proteinuria and the serum creatinine concentrations. The fall in proteinuria in the patients who survived during the study as compared to the proteinuria of those who died, contrasts with the findings of

Table VI. *Changes in the renal states after withdrawal of azathioprine*

Four patients died 1–8 mo. after withdrawal of treatment, 3 from uraemia, 1 from other causes

Renal state	No. of pats.	Observation after withdrawal (mo.) Median	Range
Normalized	0		
Improved	3	1	1–20
Unchanged	18*	6	1–35
Deteriorated	5	2	1–11

* Including 7 patients with normalized renal state at withdrawal.

Table VII. *Analysis of 18 patients who died within 84 months from the start of azathioprine treatment*

Renal state at death	No. of pats.	Duration of treatment (mo.)		Causes of death	
		Median	Range	Uraemia	Others
Normalized	0				
Improved	3	10	3–18	0	3
Unchanged	3	3	1–16	0	3
Deteriorated	12	18	2–69	7	5

Booth and Aber (4). The decrease in patients with idiopathic nephrotic syndrome is perhaps not surprising, as "minimal glomerular changes" are usually considered to carry a better prognosis than other types of glomerular diseases (9 12). On the other hand the patients with idiopathic nephrotic syndrome in the present study were either resistant to corticosteroids or "frequent relapsers" who in childhood have been reported to be resistant to azathioprine (1). Furthermore the decrease in proteinuria was also seen in patients with the clinical diagnosis of glomerulonephritis as well as in patients with histological extracapillary glomerulopathy Apparently age between 20 and 50 carries some resistance to the cytostatic treatment. The results of the present study are suggestive of some additional effect of prednisone, whether given before or during the treatment with azathioprine.

Patients with renal diseases as part of systemic connective tissue diseases have been excluded from several other studies (4 7 9) on the assumption that these patients may react favourably to the treatment. This seems to be true of some patients with lupus nephritis (3 5). In our study the patients with connective tissue diseases seemed rather resistant to therapy in comparison to the two other clinical groups. This difference between our results and those of others may be explained more advanced stages of the diseases in our lents and by the fact that also patients with iseases other than SLE were included.

The resistance to therapy in patients with elevated serum creatinine concentrations and arterial hypertension may reflect the importance of the activity and the stage of the disease for the result of the treatment

The classification of the patients according to the "summarized renal state" at the different stages of analysis represents an attempt to evaluate the clinical relevance of the alterations in serum creatinine and proteinuria. In view of the long duration of the diseases prior to the treatment, as well as of the type of histological changes, it is noteworthy that about half of the patients could be classified as normalized or improved in the 12 as well as in the 84-month analysis. Also in this classfication the importance of the variables clinical diagnosis, arterial BP and initial serum creatinine concentration was apparent, whereas the significance of the histological glomerular changes was not evident. This is probably due to the more severe criteria of alteration.

Severe deterioration of glomerulonephritis has been reported after withdrawal of cytostatic treatment (6). In our study some deterioration of the renal state was observed in 5 out of 26 patients. All these patients had renal diseases as part of connective tissue diseases.

Analysis of the patients who died during the study showed the importance only of the variable initial serum creatinine concentration. The lack of demonstrable influence of other variables may be due to the small number of patients in some of the subgroups.

Provided that the changes in the renal diseases in the present study may be ascribed to the cytostatic treatment, the fabourable results obtained with azathioprine differ from those reported in some controlled studies (4 7). This may be due to differences in the type of classification and/or differences in the duration of the treatment, which in our study was rather long. It is less likely that the severity and stages of the diseases are of importance, as these factors would be expected to diminish the chances of a favourable result in our study

ACKNOWLEDGEMENT

The study was supported by grants from Den lægevidenskabelige forskningsfond for Storkøbenhavn, Færøerne og Grønland.

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Acta med. scand. Vol. 193 pp. 493—498, 1973

CYTOSTATIC TREATMENT OF GLOMERULAR DISEASES

II. *Effect of Azathioprine on Serum and Urine Proteins*

Report from a Copenhagen Study Group of Renal Diseases

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Abstract. Urine and serum proteins have been determined by the Mancini technique in 33 patients with histologically verified glomerular diseases subjected to 12 months' treatment with azathioprine. A statistically significant decrease in the urinary excretion of albumin, IgG, IgA and transferrin was demonstrated during the treatment period. The excretion of α_2-macroglobulin and IgM was often zero or trace. Correspondingly increases of serum concentrations of albumin, transferrin and IgG were observed. Also decreases in clearance of albumin, IgG and IgA were demonstrated. The level of selectivity index before treatment could not be correlated significantly to various clinical characteristics, but the histological subgroups showed statistically significant differences. Patients with the highest selectivity generally responded best to the cytostatic treatment. During treatment certain subgroups showed increasing selectivity. This applied to patients treated with prednisone, patients without hypertension and those with membranoproliferative glomerular lesions. IgA was the protein fraction showing the greatest number of significant correlations between excretion or clearance and clinical or histological diagnosis. However, the differentiated examinations of the excretion of plasma proteins gave little more information concerning the result of treatment than did total proteinuria.

In a previous publication (3) we reported the effect of azathioprine on serum creatinine and proteinuria in patients with glomerular diseases. The present paper is a more detailed account of the changes in serum and urine protein fractions in the same patients. We also report observations on the selectivity of proteinuria, which some authors have correlated to histological diagnosis and to result of treatment in various renal dise... 6, 8 10, 14).

MATERIAL

Thirty-three patients with chronic glomerular disease treated with azathioprine were examined. The histological diagnosis was in all cases based on specimen obtained by percutaneous renal biopsy. The criteria for admission to the trial as well as the principles of the treatment have been described elsewhere (3). The characteristics of the patients are listed in Table I.

METHODS

The concentrations in serum and urine of albumin, α_2-macroglobulin, IgG, IgA, IgM and transferrin were determined at the beginning of the trial and at intervals of 2 months. Only the results from the first 12 months of treatment are reported. The proteins were determined by the immuno-diffusion method of Mancini et al. (11). Not all the 33 patients were followed through 12 months. In some of them the treatment was started before the investigation of proteins in serum and urine was systematised. In other patients the analyses were not carried out for technical reasons.

The pattern of proteinuria in the individual patient at the various times of observation was characterized by calculation of clearance (glomerular filtration rate) of each plasma protein, and of the selectivity index. The selectivity index was defined as the coefficient of regression of the line relating the logarithm of the ratio between the clearance of IgG, IgA and transferrin and the clearance of albumin to the logarithm of the molecular weight of the plasma proteins (9). This line was

validity of the selectivity index, which is also of little value in determining the etiology of the renal disease. Histological and immunological examination of kidney biopsy material is at present the best means of assessing the prognosis and etiology in the individual renal patient (7 8).

ACKNOWLEDGEMENT

This study was supported by grants from Den lægevidenskabelige forskningsfond for Storkøbenhavn, Færøerne og Grønland.

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Acta med. scand. Vol. 193 pp. 499–503 1973

FACTORS AFFECTING THE RELIABILITY OF SCREENING TESTS FOR BACTERIURIA

I. *Nitrite Test (Uritest®) Uriglox® and Dip-slide (Inculator®)*

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Abstract. All the urine specimens were examined by conventional culture, the dip-slide system, and two chemical tests for bacteriuria. The persons in the study had all followed the instructions for the chemical tests; the period of incubation in the bladder was at least six hours. The dispensary series comprised 1 264 persons from whom clean-voided midstream specimens were obtained at medical examination because of findings on screening. The hospital series included 141 patients admitted to the Renal Clinic because of findings at medical examination; urine specimens were obtained by percutaneous suprapubic aspiration and clean-voided midstream specimens were collected after the puncture. The Inculator® dip-slide showed a sensitivity of 88–100% in the two series and specificity of 97.4–100%. This high reliability agrees with the results reported for the Uricult® dip-slide. In the dispensary series the Uritest® and the Uriglox® gave sensitivity of 70.3% and 48.4% respectively and a specificity of around 99.7%. In the hospital series the figures were 94.9% and 76.3% respectively and 98.4–100.0%. With Uritest and Uriglox combined, positive results by both being required, the sensitivity was in the two series 75.0% and around 98.0% respectively and the specificity close to 100%. When E. coli or coliform rods were the only organisms involved in the bacteriuria the sensitivity rose for all the methods in the dispensary series; in the hospital series the value for Uriglox increased by 7–9% and that for the other two methods by a few per cent. A critical search through the literature on the nitrite test shows that the sensitivity was, on the whole, high in studies where an adequate period of incubation in the bladder is reported; also found satisfactory sensitivity under these conditions. In studies where no attention was paid to the incubation period the sensitivity values were low. The importance of the incubation period is also illustrated by our results. On the other hand a short incubation period does not seem to influence specificity. In our series, Uriglox showed on the whole higher specificity and lower sensitivity in comparison with other published reports. The great difference in sensitivity between the chemical tests in our two series, with better results in the hospital series, is discussed. It is concluded that there might exist factors which so far have not been sufficiently considered and which could affect the reliability of the tests. In a series of 8 314 urinary cultures the number could have been reduced by 91–92% by screening of the negative specimens (dip-slide).

This is the first of a series of papers on the results of a population study (3 998 persons) concerning the prevalence of bacteriuria and other symptoms of possible urinary tract infections, etc. The investigation included methodological studies of the reliability of some chemical tests for bacteriuria, namely the *nitrite test* (Uritest) and Uriglox, and the dip-slide test (Inculator). These methods and conventional urine culture were used in parallel at examination of 1 528 urine specimens; culture showed significant bacteriuria (> 10^5 bacteria/ml urine) in 193 specimens.

Urine specimens were collected from (*a*) persons who, because of positive screening tests, were called to appear for medical examination at a special dispensary and (*b*) persons who because of findings by medical examination, were admitted to the Renal Clinic for further investigation. In the latter series, specimens of urine were obtained both by percutaneous suprapubic aspiration and by voiding immediately after the puncture. Methodological studies under such varying conditions do not seem to have been published earlier.

The results reported here were presented in a paper read at the National Medical Convention in Stockholm in Nov 1970 and at a symposium on screening tests for bacteriuria at the National Medical Convention in Nov 1971.

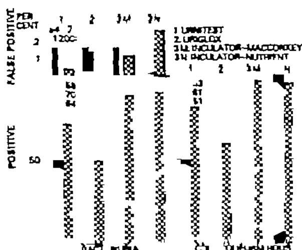


Fig 1 Sensitivity and specificity of Urintest®, Uriglox® and Inculator® in comparison with conventional urine culture showing bacteriuria (> 10⁵ bacteria/ml) and sensitivity when urine culture yielded growth of E. coli or coliform rods. The first of the narrow filled bars represents results from percutaneous suprapubic aspiration urine and the second results from subsequent midstream specimens (hospital series). The broad checkered bar represents midstream specimens collected at the dispensary. The figures indicate the number of tests that formed the bases for the calculation of the percentages marked beside the bars.

MATERIAL AND METHODS

..al questionnaire was used to check that each per- who took part in the survey had fasted and taken no d after 10 p.m. the previous evening. In the same w y information was obtained on the interval between the last micturition and the sampling of urine for examination. In the *dispensary series (a), comprising 1 264 persons,* the interval was 6 1 4 hours in two, 6 1 2 hours in one, 7 hours in one, 7 1 hours in one, and 6 hours in the rest, persons with shorter intervals were withdrawn from the series. Clean-voided midstream specimens were obtained at the dispensary in 20.. % before 9 a.m., in 58.5 % before 11 a.m., and in 83.5 % before 1.. o'clock. In the hospital series (*b*) of 141, percutaneous suprapubic aspiration of urine was performed in 77 1 before 9 a.m., in 87.1 % befor 11 a.m., and in 98.3 % before 12 clock. Clean-voided midstream specimens were obtained about a quarter of an hour after the puncture the number of suprapubic specimens was reduced to 123, since all of them were not examined by all the methods.

Suprapubic aspiration was performed by the current technique in all cases except 11 In the latter the puncture was made with relatively coarse cannula of the kind used at lumbar puncture; urine was first aspirated through the needle, thereafter a plastic tube as passed to the base of the bladder and urine was aspirated through the tube. As *urine phlegmon developed in the suprapubic tissue in* the last tw cases, this examination was discontinued. This phlegmon was attended with fever and pain for few days and was then resorbed without requiring any special measures. The conventional suprapubic aspiration caused slight macroscopical haematuria in some cases, but no other complications occurred.

The interval between the last micturition and the percutaneous suprapubic aspiration was 10..4 ± 1.61 hours for the whole series, *10.59 ± 1 44* hours for the persons with significant bacteriuria, and 9.89 ± 1.84 hours for those without significant bacteriuria. The number of minutes are here expressed in decimals.

Persons who were undergoing drug treatment or had glucosuria (diabetes etc.) were excluded from the series. Antiseptics were not used in cleaning the region around the orifice of the urethra.

Conventional urine culture was carried out at the Bacteriological Routine Laboratory of the University Hospital. The urine specimens were refrigerated immediately and kept at low temperature until the bacteriological examination. Examinations by the other methods were carried out in the dispensary laboratory The dip-slide of the commercial type, Inculator had two media on its slide, MacConkey medium and nutrient medium; to the former has been added substance for inhibition of growth of contaminants. After inoculation and storage in thermostat at 37°C for 18 hours, the dip-slide results were read by reference to the chart supplied by the makers.

RESULTS

Fig. 1 illustrates the results in the two series, comprising urine specimens obtained from the in-patients by suprapubic aspiration (123 persons), clean- oided midstream specimens collected after the puncture (141 persons) and midstream specimens voided at the dispensary (1 264 persons).

Urine culture showed significant bacteriuria in 59 70 and 64 cases, respectively for specimens with only E. coli or coliform rods the comparable figures were 53 61 and 51 Bacteriuria of coliform type was recorded for 3 4 and 3 cases, respectively The numbers of positive chemical tests and dip-slides are gi en as percentages of all specimens with bacteriuria, E. coli and coliform rods, respectively

Table I shows the remaining cases of bacteriuria of other types.

No or insignificant bacteriuria on urine culture was recorded in 64 71 and 1 200 cases, respectively The numbers of false positive chemical tests and dip-slides are given as percentages of these figures.

The highest sensitivity was found in bacteriuria with E. coli and coliform rods, namely 100 % for the Inculator in suprapubic and midstream speci-

Table I. *Remaining cases with significant bacteriuria on culture and bacteria other than E. coli or coliform rods: positive chemical tests or dip-slide (MacConkey and nutrient media)*

Significant bacteriuria on culture		No. of positive chemical tests and dip-slides			
	No. of urine			Dip-slide (Inculator)	
Type of infection	specimens	Uritest	Uriglox	MacConkey	Nutrient
Enterococcus	10	3	1	5	8
Proteus species	5	4	1	5	5
Staphylococcus	7	4	1	1	5
Pseudomonas pyocyaneus	3	3	0	3	3
Mixed					
Enterococcus and E. coli	1	0	0	0	0
Enterococcus and Proteus or Staphylococcus and coliform rods	2	2	1	2	2
Total	28	16	4	16	23

mens from the patients in the hospital series, 80.4% for the Uritest, and 58.8% for the Uriglox. Fig. 1 does not include the results for the following combinations of chemical tests: Uritest or Uriglox positive (100.0 100.0 and 84.3% respectively) and Uritest and Uriglox positive (81 1 86.9 and 54.9% respectively). If the dip-slide and a chemical test are combined and the requirement is that both should be positive, the result will be determined by the lower sensitivity of the chemical test, if the requirement is that either should be positive, the result will be determined by the higher sensitivity of the dip-slide.

When a comparison was made with the result of urine culture irrespective of bacterial species, that is, significant bacteriuria including all bacterial species present in the urine (Fig. 1 left part of the diagram) the sensitivity showed throughout lower figures, namely Inculator (a crage for MacConkeys and nutrient medium) 96.6 and 98.0% in the two groups of the hospital series and 88.3% in the dispensary series, Uritest 94.9 and 95 7% in the former and 70.3% in the latter and Uriglox 76.3 and 81 4% in the former and 48.4% in the latter series. Fig. 1 also shows the number of false positive chemical tests and dip-slides. The alues are, on the whole, lowest for the two chemical tests, which ha e a slightly higher specificity than the dip-slide, namely 98.4%–100.0% and 97 4%–100 0% respectively

The left part of the diagram does not include the sensitivity for the following combinations of the two chemical tests. With the requirement that either Uritest or Uriglox should be positive, the sensitivity was 98.3% and 97 1 respectively in the two groups of the hospital series and 75 0% in the dispensary series, with a positive result required for both, the figures were 72.9 % and 80.0% in the former and 43.7% in the latter series. Under the same conditions the specificity in the respective series was for each combination of tests 96.9% and 98.6% in the former and 99 6% in the latter series and 96.9% and 98.6% in the former and 99.9% in the latter series.

Table I shows the sensiti ity of Uritest and Uriglox, and of Inculator in significant bacteriuria caused by bacterial species other than E. coli and coliform rods.

From a dispensary 8 314 urine specimens were sent to the University Hospital's Bacteriological Routine Laboratory Out of these, only 765 revealed significant bacteriuria (9.2%) By screening off the negative specimens (dip-slide) the number of urinary cultures could ha e been re d ced by some 91%

COMMENTS

This paper deals mainly with the percentage reliability of two chemical tests and dip-slide in comparison with conventional urine culture. For practical reasons we apply the postulate that the results of the culture are correct. The following example shows that this is probably not alw y the case.

In the tw series of percutaneous suprapubic aspiration of urine and of collection of clean midstream specimens voided after the puncture, both specimens produced growth in 4 cases, but the counts were less than 10^5 bacteria ml in the cultures. Thus significant bacteriuria was not present. Both chemical tests and dip-slides were negative in all the specimens. In another case culture of suprapubic urine yielded bacterial growth, the count being less than 10^5 ml, whereas the chemical tests and dip-slide showed significant bacteriuria. In the specimens voided after the puncture culture as well as the chemical tests and dip-slide showed significant bacteriuria. It is thus probable that culture on suprapubic urine produced too low bacterial count.

No further comparisons will be made here bet een the presence of bacteriuria in suprapubic urine and in voided urine from the same person.

The object of this study was to examine the reliability of the methods of detecting bacteriuria on screening. Therefore the main interest concerns the results obtained in bacteriuria defined as a total count exceeding 10^5/ml, irrespective of bacterial species, and in non-bacteriuria by the said criteria. In the dispensary series the incubation in the bladder a eraged 6 hours and 5 minutes (6–7 hours). The dip-slide (MacConkey) ga e the highest sensitivity 87.5% and satisfactory specificity 98.9%. Uritest and Uriglox showed higher specificity 99.7% and 99.8% but unsatisfactory sensitivity 70.3% and 48.4% respectively. In the hospital series there was good agreement between the results of the methods used for suprapubic urine and voided urine and the sensitivity values were higher. The latter rose for Uritest by 25 to 95.0–96.0% for Uriglox by an a erage of 29% to 76.3–81.4% and for Inculator by about 7 to 94.9–95.7 (MacConkey's medium) and by 10% to 98.3–100.0% (nutrient). Specificity decreased slightly for all the methods, except for dip-slide with nutrient. In the hospital series the incubation period in the bladder was in most cases much longer than in the dispensary series, a eraging about 10 hours, as against 6 hours.

The possible explanation of these differences may be discussed. Insofar as correct information was gi en, the incubation period in the bladder was at least 6 hours. This length of time is sufficient for the nitrite test and Uriglox. All the persons in the dispensary series had, according to information also followed the other instructions for the Uriglox test. Purely hypothetically the possibility may be mentioned that the patients experienced the situation as more serious when hospital care was necessary and therefore followed the instructions particularly carefully or ga e adequate information about having carried them out. The number of specimens in the dispensary series, 1 64 was much greater than in the two hospital series, 123 and 141 respectively and the selection of persons for the study was made on different grounds for the two series, namely screening without urine culture and medical examination including urine culture respectively. The percentage of bacteriuries will therefore be high in the hospital series, as will the relative number of specimens with pathogenic bacteria. Since it does not seem possible to explain why the results of the methods used were better in the hospital series than in the dispensary series, the question concerning the cause of the differences will be left open.

For significant bacteriuria (> 10^5 organisms/ml) with in olvement of E. coli and coliform rods alone—the latter were present in 3% of the specimens—the results, in comparison with those reported in the foregoing, were as follows. In the dispensary series the Uritest values rose by 10.1% to 80.4% the Uriglox values rose by 10.4% to 58.8% and the dip-slide values rose by about 6% to about 95%. In the hospital series the values rose by about 1–3% to 96.2–98.4% for Uritest, by 7–9% to 84.9–88.5 % for Uriglox, and by 4–6% to 100.0% for the dip-slide. Accordingly when these were the only bacteria in olved, the sensitivity increased for all the methods, notably for the chemical tests in the dispensary series.

The demonstrated effect on the reliability of the chemical tests performed under arying conditions, which are not expected to influence the results, is of course of great practical importance in screening. Our results, which differed between the hospital series and the dispensary series, should also be considered in comparing data in published reports, especially as many authors do not give essential details on the conditions under which the tests were made.

Published data on the reliability of the Uricult dip-slide method confirm the good results obtained by us with the Inculator when the inoculated dip-slide is incubated at 37°C for 18 hours. Arneil et al. (1) found, for instance, no false negative

and one false positive Uricult result in a series of 141 urine specimens with positive pour plate. Wille and Winter (16) also found a good correlation between the Uricult test and pour-plate culture, usually after incubation at 37 C for 16 hours, their series comprised more than 2 500 specimens.

Results obtained by Griess nitrite test, reported in papers with data showing that the bladder incubation (4 hours) was in all probability adequate, are in good agreement with our results. Some reported figures are sensitivity 76.7% (specificity 96 6%) in a series of 624 urine specimens, including 30 with significant bacteriuria on three cultures (5) 57 7% (97 0%) in a series of 557 hospital patients, including 142 with significant bacteriuria on one culture (14) DeShan et al. (4) obtained good results, 90.5% (99.3%) in a series of 608 specimens, including 42 with one positive culture, although the bladder incubation was stated to be at least 2–3 hours. Other reports without clear data on the incubation period show much poorer results, for example sensitivity 35.0% (specificity 99 1%) in a series of 3 000 specimens, including 184 with bacteriuria on one culture (9), 39 8% (99.9%) in a series of 2 354 specimens, including 118 with either one specimen yielding more than 10^4 bacteria/ml or two specimens yielding more than 10^3 on culture (13), and 36.5% (99.5%) in a series of 887 specimens with bacteriuria on one culture (15).

The importance of carrying out and recording the instructions for the period of incubation in the bladder is borne out by the following, as yet unpublished, experience from the Renal Clinic. Because of an error in the routine the patients handed in urine specimens at the medical examination without previous special instructions for the nitrite test (Urnitest) and without information as regards the incubation period. The series comprised 5 039 specimens, including 765 with bacteriuria on one culture and the rest without growth or less than 10^3 bacteria/ml Sensitivity was 31.5% and specificity 99 0% A similar error was made in a small part of our present population study The series comprised 707 specimens, including 36 with bacteriuria on one culture, sensitivity was 25 0% and specificity 99 9%

Thus, unless due attention is paid to the required incubation period for urine in the bladder the sensitivity of the nitrite test will be low as expected the specificity on the other hand, will remain good.

In our series the Uriglox test showed, on the whole higher specificity and lower sensitivity than those reported in the literature. Some of the published sensitivity figures, however are based on relatively small numbers of cases of bacteriuria on culture. The originators of the method, Scherstén and his collaborators, report 95% and 96% in 83 and 97 cases, respectively (3 6). Other reported figures are 91.3% in 103 cases (7) 85 7% in 28 cases (10) 81 0% in 16 cases (2) 79 0% in 103 cases (11) 70.0% in 21 cases (12) and 67.0% in 21 cases (8)

No attempts will be made here to analyse the causes of the fairly great differences between the published figures and between our and other authors results. The discrepancy in the results between our two series suggests that there may exist factors which up to now have not received sufficient attention and which affect the reliability of the Uriglox test, as well as of the nitrite test.

ACKNOWLEDGEMENTS

This study was supported by grants from Malmöhus Local Social Insurance Office and AB Gambro, Lund

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Acta med. scand. Vol. 193, pp. 505–509 1973

FACTORS AFFECTING THE RELIABILITY OF SCREENING TESTS FOR BACTERIURIA

II. *Dip-slide: False Positive Results Following Postal Transport and False Negatives Owing to Incubation at Room Temperature*

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Abstract In screening programme 1 488 persons inoculated dip-slides (Inculator®) in their homes and posted them to central laboratory where they were read after incubation in thermostat for 18 hours at 37°C. A high frequency of significant bacteriuria as recorded Despite published statements to the contrary there were reasons to presume that bacterial growth during the transport at the current outside temperature created large number of false positive results. At the medical examination of the same persons few weeks later the inoculated dip-slides were incubated in thermostat without delay The prevalence of significant bacteriuria was reduced by 50–80%. Computer analyses showed that, in many cases, initial non-significant bacteriuria probably increased during the transport and became significant, thus giving false positive results. This presumption as borne out by the results of the laboratory tests, at which series of inoculated dip-slides were read after immediate incubation in thermostat and after storage for 24–72 hours at room temperature, ith subsequent incubation in thermostat, respectively The number of false positives increased ith the duration of storage at room temperature before incubation. The laboratory study also showed that storage of inoculated dip-slides for 24 hours at room temperature gives smaller number of positive results than does incubation in thermostat for 18 hours (false negatives) The literature contains conflicting data on this point. A technique has been elaborated to provide solution to the problem of transporting inoculated dip-slides.

Incubation at 37 C for about 18 hours immediately after the inoculation of dip-slides with urine produces results that agree well with those obtained by con entional urine culture, according to unanimous reports in the literature (1). As regards the influence on the reliability of the dip-slide technique, however opinions differ on the following questions. (*a*) Can storage of inoculated dip-slides at room temperature for 4 hours replace incubation at 37 C for 18 hours and (*b*) can dip-slides be inoculated, e.g. in the home or at the doctor's office, and then transported by post for one or several days at the current outside temperature to a central laboratory where they are read after incubation at 37 C, without the risk of false negative results? These procedures are recommended in the literature and in the folders supplied with the commercially manufactured dip-slides.

The latter procedure was used in screening 3 998 persons for bacteriuria with the Inculator dip-slide, which consists of a glass slide coated w th two media, MacConkey's medium and nutrient agar Significant bacteriuria, more than 10^5 bacteria/ml of urine, was considered to be present in 2.5–2.4% of the men and in 10.1–12.1% of the women for the respecti e medium in our population study from 1969–1970 In comparison with figures published by othe authors, our figures were too high. At medical examination of those who had bacteriuria on screening, only 0.4% of the men and 5.2% of the women had significant bacteriuria on con entional urine culture. Accordingly a large number of dip-slides produced false positive results on screening.

The problem is illustrated by an analysis of the screening results in comparison with the results from the medical examination of urine specimens that were not mailed, i.e. immediate incubation at 37 C of the inoculated dip-slides, and by laboratory studies on the effect of storage at room temperature for 24–72 hours.

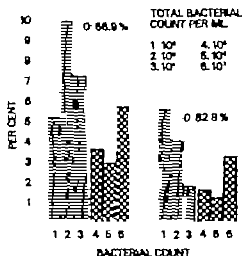


Fig. 1 The left part show the dip-slide (Inculator®) results from 1 488 persons who inoculated the slides with clean-voided midstream urine in their homes and posted them to the laboratory where the results were read after incubation for 18 hours in thermostat at 37°C. The right part show the results of the second examination of the same persons, when the inoculated dip-slides wer without delay incubated in thermostat. The results were read mainly by reference to the chart supplied by the makers, graded 0–10⁶ bacteria ml, corresponding to our gradings 0–6 4–6 indicate significant bacteriuria.

METHODS AND MATERIAL

Inculator dip-slide was used According to the chart ..ied by the makers, gradings 1–6 correspond to 10' 10⁶ bacteria ml of urine At least one colony was required for grade 1 however the grading on the chart does not include any colony Grades 4–6 correspond to significant bacteriuria, that is, more than 10' bacteria ml of urine

Urines from 1 488 persons ere examined by screening tests and at medical examination about 1 2 weeks later At the screening the persons themselves inoculated the dip-slides in their homes and posted them to the dispensary's laboratory here the results ere read after incubation for 18 hours in th.rmostat at 37°C At the re-examination the dip-slides were incubated immediately after the inoculation On grounds of space, only the results of the reading of MacConkey medium will be reported for these as ell as for the laboratory studies, the nutrient medium gave, on the hole identical results

Fig. 2 shows the different series and he number of examined specimens in the laboratory studies Eighteen urine specimens from patients with bacteriuria not under treatment ere diluted ith urine from healthy persons In order to obtain suitable numbers of bacteria per ml; bacterial counts lower than 10⁷ seemed to be of special interest In this connection The series ere di ided as follows (*a*) incubation for 18 hours at 37°C (*b*) incubation in thermostat for 18 hours after storage for 24 48, and 72 hours, respectiv ly at room temperature (around 22°C), the results being read every day and daily readings during stor ge at room temperature for up to 72 hours, thus, in this series the dip-slides ere not incubated in a thermostat Since the results ere read repeatedly in the latter two series, the number of observations after .g 24 hours at room temperatur were 133 and 183 respectively and after 72 hours 32 and 22, respectively The important finding here is not the absolute differences but the trend under different test conditions. Gram-negative bacteria predominated in cultures on the studied urines. The relationship between the bacterial species and the time of development of visible colonies on the dip-slide is outside the scope f this study

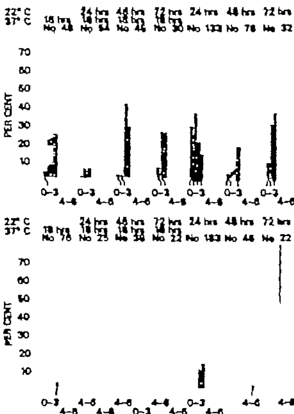


Fig 2 Results from laboratory studies f the influence of incubation for 18 hours at 37°C and storage at room temperature for 1 to 3 days without or with subsequent incubation in thermostat on the outcome of tests ith dip-slides (Inculator®) inoculated ith diluted urine from patients with bacteriuria The upper part of the diagram refers to the series with non-significant bacteriuria after incubation of the dip-slides in thermostat, and he lower part to those with significant bacteriuria (excluding 4% w th non-significant bacteriuria among other positive specimens in the different series) The number of bacteria was graded according to the makers chart from 0 to 6, corresponding to 0–10 bacteria ml 4–6 indicate significant bacteriuria.

RESULTS

1 Bacterial counts of dip-slides inoculated at screening in the field mailed and incubated for 18 hours at 37 C and inoculated and incubated without delay respectively

This computer study of clean-voided midstream urines from 1 488 persons was carried out in co-operation with Mr Jan af Ekenstam. The report is concerned with MacConkey's medium alone. Fig. 1 shows the following percentage distribution of gradings 0–6 of ( ) dip-slides inoculated by the persons themselves in their homes and mailed to the laboratory where the results were read after incubation in a thermostat for 18 hours and (*b*) dip-slides inoculated with urines from the same persons at examination at the dispensary and immediately incubated for 18 hours in a thermostat before reading. The percentages for the latter series are given in parentheses.

No colonies were noted in 66.9 (82.8) % non-significant bacteriuria was present in 20.9 (10.9) % and significant bacteriuria in 12.2 (6 3) % The conclusion is that the difference was wholly or mainly due to bacterial growth during transport by post for one or several days at the current outside temperature. Excluding the uncertain grading 1 (10^3), the following figures for grades 2 and 3 were obtained: 17 2 (5.9) % These percentages suggest a hea y growth under the conditions of screening within the range of non-significant bacteriuria increasing to significant bacteriuria in 6 % of the persons investigated.

2. Labor tory studies on the ffect of storage at oom temperature of inoculated dip-slides for 24–72 hours with or without subsequent incubation for 18 hours at 37 C

Fig. 2 shows the results. The upper part of th diagram refers to the series in which non-significant bacteriuria was noted after incubation in a thermostat immediately after inoculation the series grouped in the lower part of the diagram had significant bacteriuria *under the same test* conditions It should be noted however that the agreement between the parallel specimens is not complete in the latter series, in that 4 of the dip-slides produced non-significant bacteriuria (10^4)

The urine specimens that belonged to the ser without significant bacteriuria afte incuba for 18 hours in a thermostat had bacterial counts exceeding 10^3/ml in 7 20, and 40 % when the inoculated dip-slides were stored at room temperature for 4 48 and 7? hours and then incubated. These results reflect the effect of transport by post on our screening specimens. Storage at room temperature for 48 and 72 hours caused significant bacteriuria (false positi es) in 15 % and 2. % respectively of the dip-slides Storage at about .2 C for 24 hours, on the other hand, did not cause bacterial growth corresponding to significant bacteriuria, that is, no false positives

In the other series, in which 96 of the dip-slides were positive after incubation for 18 hours in a thermostat, all the specimens showed significant bacteriuria after storage for 24 48 and 72 hours, respectively at room temperature nd subsequent incubation in a thermostat Of greater interest in this connection however is the fact that only 75 % of the positive dip-slides showed counts exceeding 10 after storage at room temperature for 24 hours. Accordingly under these conditions the bacterial growth was sparser than after incubation for 18 hours in a thermostat, which gave a 21 % higher incidence of bacteriuria in the specimens All the specimens showed significant bacteriuria after storage for 48–72 hours at room temperature

COMMENTS

At the start in 1969 of our screening programme which included inoculation of dip-slides in the field and transport by post to our laboratory (dispensary) for incubation at 37 C before reading, many reports in the reliability of this technique could be found in the literature As early as 1965 Mackey (7) in a paper on laboratory diagnosis of infections of the urinary tract in general practice by means of a dip-inoculum transport medium, stated: After inoculation the outfit may be returned by post to the laboratory where a *clinically accurate* able count can be assessed by inspection after overnight incubation. The statement was briefly elucidated by the information that one spoon was incubated immediately and that the second and third spoons were kept at room temperature for 24 and 48 hours re ely before incubation no significant colony count were obtained.

possibility of sending inoculated dip-slides by post to a central laboratory was mentioned in three papers: A modified dip-inoculum transport medium for the laboratory diagnosis of infections of the urinary tract. On arrival at the laboratory and a delay is of no consequence, the unopened container is incubated and the slide examined on the following day" (10) the slide is sent to the laboratory if necessary by post. It is particularly useful in general practice if specimens have to be sent by post (6). The slide cultures may be incubated and read in the field or mailed to a central laboratory for reading, identification and sensitivity testing of isolates (5).

Our results show a large number of false positive screening specimens, in all probability attributable to growth of bacteria on the dip-slides, which were inoculated in the homes with urines from persons who had non-significant bacteriuria. This conclusion was confirmed by the findings in the laboratory studies. Results that agree with ours were reported in 1970 (1) from a small series. Eighty-three inoculated dip-slides (Uricult®) and urine specimens diluted with Calcium-Triplex® were sent by post between Nov and March (me of transport cars and ferries) to a central lab ratory The transport took 1–3 days. cult ilded bacterial counts exceeding bacteri n 41 specimens dip-slides showed ificant ria in 40 of these specimens (false neg ts in 2.7 %) Thirty-seven cultures yiel wth or bacterial counts below 10⁴ one showed significant bacteriuria (more th bacteria/ml). Five cultures produced bac unts exceeding 10⁴ to less than 10⁵ two di es showed significant bacteriuria. Thus the p rtion of false positive dip-slides was 7.3% O the basis of six postal specimens, Arneil et al. ( ) all attention to the unreliability of the results f om delayed or posted specimens.

So it is evide t that inoculated dip-slides cannot be sent by post at the current outside temperature to a central laboratory for subsequent incubation at 37 C without the risk of false positive results.

Arneil et al. (2) reported that, compared with pour-plate controls at 37 C, almost identical results were obtained from 140 consecutive urine specimens cultured on dip-slides (Uricult) incubated for 18 hours at 15–18 C, 18 hours at 37 C, 18 hours at 15–18 C plus 24 hours at 37 C. All three groups of dip-slides gave identical results in 50 positive cultures, whereas a false positive result was obtained in one out of 90 negative specimens. Wille et al. (11) obtained discrepant results with dip-slides (Uricult) incubated for 16–24 hours at 21 C and with those incubated for 16 hours at 37 C. The difference was most pronounced for low and borderline germ counts (10^4–10^5/ml) The bacterial count was always lower in the specimens incubated at room temperature. Other authors (8, 9) have also obtained identical results and emphasize that incubation at room temperature alone will, to a great extent, miss Gram-negative bacteria, in particular and thus produce false negative results.

The laboratory results reported here confirm the view that incubation at room temperature for 24 hours gives less reliable results than does incubation at 37 C for 18 hours.

The different numbers of observations in the studied series do not allow any conclusions in terms of absolute figures, but they illustrate the trend, namely that storage at room temperature for 1 to 3 days (transport by post, etc. before incubation) can create false positive dip-slide results in non-significant bacteriuria. Significant bacteriuria is diagnosed less often after storage at room temperature for 24 hours than after incubation in a thermostat at 37 C for 18 hours. This means that the former procedure can produce false negative results.

The relationship between the bacterial species and the time of development of visible colonies on the dip-slide is outside the scope of this study

The following technique has been elaborated to provide solution to the problem of transporting inoculated dip-slides: incubation of inoculated dip-slides for 18 hours at 37°C at the doctor's office or the technician's laboratory drop of an inhibitor of bacterial growth is added; these dip-slides can be sent by ordinary mail to laboratory to be read without any risk of false positive results.

ACKNOWLEDGEMENTS

This study was supported by grants from Malmöhus Local Social Insurance Office and AB Gambro, Lund.

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Acta med. scand. Vol. 193 pp. 511–514 1973

FACTORS AFFECTING THE RELIABILITY OF SCREENING TESTS FOR BACTERIURIA

III. Sensitivity and Specificity of the Uriglox® and Various Nitrit Tests Including a Study of the Reliability of the Uritest® as Assessed by the Screened Persons

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Abstract. Urine specimens have been tested in parallel by conventional culture, Uritest®, BM Test Nitrite®, BM Test 4®, Griess test, and Uriglo ®. The following values were obtained for the sensitivity and the specificity (figures within parentheses) of the chemical tests: for the nitrite tests in the said order 74.0 (99.7)%, 71.2 (99.8)%, 69.9 (99.7)%, 76.7 (99.6)%, and for Uriglox 52.1 (99.8)%. The variations with respect to positive, false positive, and false negative specimens were analysed. W based our comparisons on the results of the cultures and of each of the ch ical tests and studied the results from the other spec ens. The figures seem to be of interest, in that they give an idea of the numerical magnitude of the afore mentioned variations. A similar comparison was made between the results from the arious chemical tests in the screening of 3 988 persons. 3 731 urine specimens were examined by the Uritest, partly by the subjects themselves in their homes and partly at our laboratory. Out of 3 592 tests assessed as negative at home, 99.6% were also negative at the laboratory and 0.4% were positive. Out of 139 specimens assessed as positi at home, only 53.2% were positive at the laboratory. The test may therefore be suitable for excluding bacteriuria by the person at home, but there is the risk of over-diagnosis. It should be stressed, however that at most 70% of the bacteriuric cases in our series were picked up by the ni trite tests.

In a previous paper (2) we reported the results of the following parallel tests of urine specimens from 1 264 persons in connection with medical examination: con entional culture, dip-slide, Uritest, and Uriglox. These persons are included in the present series, comprising 1 459 persons. Each specimen was examined by conventional culture, Uriglox, and the following nitrite tests: Uritest, BM Test Nitrite, BM Test 4 and Griess test. The series presented here thus concerns mainly a comparison between different nitrite tests.

We also report here results of screening by the said chemical tests of urine specimens posted to our laboratory by 3 988 persons. 3 731 of them carried out the Uritest at home on urine specimens which were later examined at the laboratory. Con entional urine culture was not used in these series.

MATERIAL AND METHODS

Clean-voided midstream urine specimens ere collected at the medical examination; the interval between the last micturition and voiding was at least 6 hours.

At the screening each subject collected morning urine at home in tube hich contained drops of 10% thimerosalat solution. The specimens ere sent by post to our laboratory. The subjects also sent written statements on the result of their ow assessments of the Uritest made on the urine specimens.

Only those ho replied in the affirmative to the question hether they had followed the instructions for the chemical tests ere included in the series. Cases of hyperglycosuria due to diabetes or renal glycosuria ere excluded.

Griess reagent as prepared at the laboratory. For the other methods commercial chemical tests ere used.

One of us (J. E.) carried out computer analysis of the material. For further details the reader is referred to the section on Material and methods in previous paper in this series (2).

RESULTS

Table I shows the results of conventional urine culture and chemical tests on 1 439 urine specimens collected at medical examination. Bacteriuria was diagnosed by culture in 5.1%, by the Uritest in 3.8%, by the BM Test Nitrite in 3.6%, by the BM Test 4 in 3.5%, by Griess test in 3.9%, and by the Uriglox in 2.6% of the specimens. Sensitivity calculated as the percentage of the total number of positi e res cl

It seemed to be of interest to analyse the variations with respect to positive, false positive, and false negative specimens, basing the comparison on the results of the cultures and of each of the chemical tests. Figures thus obtained would help to give an idea of the numerical order of magnitude of such variations. In the present studies we have not analysed the extent to which variations occur between the individual subjects in the different tests.

Similar comparisons were made in the series of 3 988 persons who were screened by chemical tests alone. The results agree with those obtained in the previous series (2) as regards the chemical tests compared with one another

Out of 3 592 nitrite tests (Urnitest) assessed as negative by the subjects in their homes, 14 parallel tests made at the laboratory or 0.4% were assessed as positive. This degree of safety is considered to be satisfactory On the other hand, the parallel tests were positive in only 53.2% of 139 tests assessed at home as positive. This is an unsatisfactory figure which indicates that it may not be suitable to leave the checking of treatment in the hands of the patients themselves.

It should be noted, however that the nitrite tests in our series picked up at most 73% of the bacteriuric cases when the tests were made at a dispensary despite the fact that we included only persons who stated that they had followed the instructions for the chemical tests.

ACKNOWLEDGEMENTS

This study was supported by grants from Malmohus Local Social Insurance Office and AB Gambro, Lund.

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Acta med. scand. Vol. 193 pp. 515–523 1973

IMPROVED DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION BY FREQUENT SERUM ENZYME DETERMINATIONS

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Abstract. Frequent and careful enzyme analyses in patients admitted to a coronary care unit (CCU) have resulted in an improved diagnostic discrimination in the borderline between angina pectoris and acute myocardial infarction—i.e. the intermediate coronary syndrome. Changes in the serum enzyme values with a course like that for AMI but within the normal value were found in about 10% of the patients admitted to the CCU. Some myocardial infarction courses were prolonged and showed several GOT peaks, especially when status anginosus occurred before admission. It is suggested that the patients belonging to the intermediate coronary syndrome have minor myocardial infarction and these patients are of special interest when discussing secondary preventive measures.

The determination of cell enzymes in the serum has become an indispensable diagnostic tool in the coronary care unit. A more careful interpretation of the results of enzyme determinations, especially near the normal range, might increase the clinical value of these laboratory tests.

At the coronary care unit of Serafimerlasarettet an attempt to improve the diagnostic accuracy in acute central chest pain is an object of special attention (11). The aim of the present study has been to study the effects of more frequent serum enzyme determinations in relation to the diagnosis of acute myocardial infarction. Thus a more distinct separation of the clinical condition in the area between the clearly established acute myocardial infarction and the common attack of angina pectoris can be obtained.

MATERIAL AND METHODS

One hundred and ninety-one patients, 118 men and 73 women, were investigated after admission to the coronary care unit (CCU). The guiding policy for admitting the patients has been the fulfilment of at least one of the following criteria. 1) Central chest pain lasting for more than 15 min within the last 48 hours. 2) Frank pulmonary oedema without previously known valvular lesion, uraemia or intoxication. 3) Shock without suspicion of acute hypovolaemia or intoxication. 4) Syncope with suspect electrocardiographic evidence of acute myocardial infarction. 5) Intractable angina pectoris.

Blood samples for enzyme analyses were drawn at admission and four more times within at least the first 24 hours (before the meals). The blood was kept at 0°C and serum was separated within an hour. Centrifugation and storage were performed at +4°C. The blood samples were analysed once a day using a Reaction Rate Analyzer (LKB 8600) connected to an evaluation unit (Optilab, Bo Philip Instrumentation, Stockholm). Reagents for aspartate aminotransferase (GOT), alanyl aminotransferase (GPT) and lactate dehydrogenase (LD) came from KABI AB, Stockholm. Heat-stable lactate dehydrogenase (LDT) was measured as LD after preincubation of serum and LD reagents except for α-ketoglutarate at 65°C for 30 min. Creatine kinase (CPK) was determined by using Monotest kit (Boehringer Mannheim, Western Germany). Normal values for the enzymes used are summarized in Table I.

The diagnosis of acute myocardial infarction (AMI) was verified by ECG changes (10) or by an increasing GOT value with a maximum of more than 40 U l (35°C) and a CPK peak followed by a peak of heat stable LD.

The diagnosis of intermediate coronary syndrome (ICS) has been given to patients showing the same type of GOT

Table I. *Normal values*

Enzyme	Normal range	Borderline	Units
GOT	10– 35	35– 40	U l (35°C)
GPT	10– 35	35– 40	U l (35°C)
LD	100–350	350–400	U l (35°C)
LDT	50–250	250–300	U l (35°C)
CPK	10– 50	50– 80	U l (25°C)

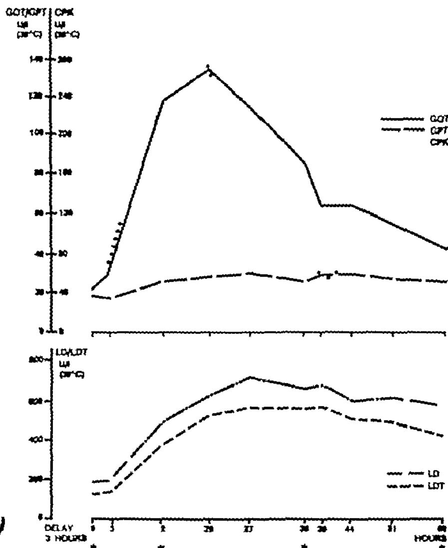


Fig. 1. Classical enzyme curves in a 42-year-old man (case 1) with acute myocardial infarction. * = time for enzyme determinations according to the ordinary routine.

and LD curves as seen in AMI but never exceeding the upper normal value (GOT 40 U/l). The difference between the highest and lowest GOT value should be more than 10 U/l without changes in GPT and with no CPK curve typical of heart muscle damage. None of these patients had subsequently electrocardiographic evidence of typical acute myocardial infarction.

The diagnosis observation (OBS) was given to the rest of the patients fulfilling the criteria for admission to the CCU but having no signs of AMI or ECG, normal constant enzyme values or other types of enzyme pattern.

RESULTS

The variations in single enzyme analyses is demonstrated by the GOT results in the observation group in Table II. 69% of the patients in this group (84 patients) had a difference between the highest and the lowest GOT value of less than 15 U/l (35°C) during the first 24 hours. The coefficient of variation for such a GOT determination is 3% ($n = 18$, $M = 33.7$ U/l, S.D. = 1.0 U/l).

Acute myocardial infarction was found in 88 patients (46%) and in 19 patients (10%) the GOT values were within the normal range but showed an enzyme pattern as found in acute myocardial infarction. This condition is here defined as the intermediate coronary syndrome (ICS) (5).

Our routine for serum enzyme determinations includes GOT, GPT, LD and LDT at admission and then on the next three mornings. The result of more frequent enzyme analyses in the present study as compared to the ordinary routine is summarized in Table III. Fifteen patients from the observation group (ordinary routine) had enzyme

Table II. *Differences between the highest and the lowest value of GOT during the first 24 hours*

Difference range (U/l)	AMI (...)	ICS (...)	Observation cases ( )
1–5	—	—	37
6–14	—	37	32
≥15	94	63	4
Not valid	6[a]	—	27[b]

Died during the first hours in the CCU
[b] Enzyme curves of other types: acute alcoholic enzyme curve, backward failure, liver diseases etc.

Table III. *Diagnosis of AMI by using frequent enzyme analyses and a comparison with the ordinary routine*

Intervals of analyses during the first 24 h	AMI	%	ICS and susp. inf.	%	Observation cases	%
Frequent (5/24 h)	88	46	19	10	84	44
Ordinary (2/24 h)	86	45	6	3	99	52

curves within the normal values but similar to AMI curves, giving them the diagnosis of the intermediate coronary syndrome. At the same time two of the patients diagnosed as suspected AMI according to the ordinary routine were thus diagnosed as AMI.

Frequent serum enzyme analyses during the first 4 hours gave a more detailed pattern of the pathophysiological course of heart muscle damage. This is illustrated by the following figures and their corresponding case reports.

Figs. 1 and 2 show typical AMI curves.

Case 1. A 42 year-old man with history of myocardial infarction, treated for hypertension. Three weeks before admission onset of dull ache in the chest and onset of dyspnoea even at rest. On the day of admission feeling of constriction with pain in the chest, which radiated to his left arm. There was sweating and vomiting. At admission BP 170/105 heart rate 76/min. No rales. The ECG 3 hours from onset: acute inferior myocardial infarction.

Case 2. A man aged 72, previously in good health. Sudden chest pain with radiation to both arms and to the jaw. Some clouding of his mind and profuse sweating. After delay of 7 hours he was admitted to the CCU. At admission the ECG showed an anterior myocardial infarction. The patient died 28 hours later and at autopsy the diagnosis could only be verified by macroscopy.

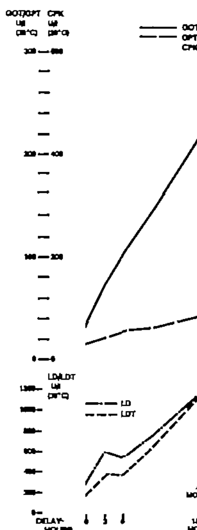


Fig. 2. The start of the enzyme curves in patient (case 2) who died 28 hours after onset of chest pain. Symbols as in Fig. 1.

Fig. 3 demonstrates how a heavy chest pain without enzyme liberation turned into a classical AMI.

Case 3. A man, 58 years old with history of recent diffuse uncharacteristic pains in the epigastrium and also in the lower part of the chest, retrosternally. The patient came to the CCU because of feeling of big stone over the chest. During the following 24 hours he was without pain and he was sent home. T hours later he returned with new pains, radiation to both arms and sweating. The ECG was unaltered for the first 4 hours. Thereafter both the ECG and the enzyme curve showed classical pattern.

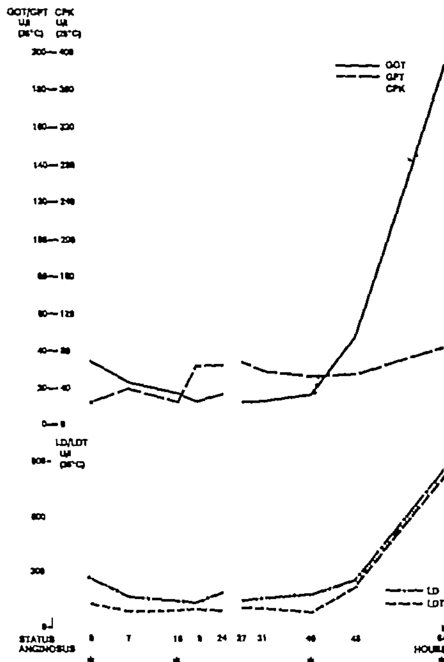


Fig. 3. Enzyme curves in a patient (case 3) with one day preinfarction angina. Symbols as in Fig. 1.

A status anginosus may culminate in an AMI. Fig. 4 shows that frequent enzyme analyses may reveal several enzyme maxima, thus explaining long-lasting pain and giving a better measure of the size of the necrotized muscle mass.

Case 4. A man, 61 years old. After a big dinner he was uncomfortable for 2 days and at the end of the second day he suffered repeated retrosternal pains. He first interpreted the symptoms as indigestion but when the pain became more severe he came to the CCU. At admission he vomited and sweated profusely. In the CCU he had diffuse pains for several days.

The transaminase values may sometimes be difficult to interpret (1). Fig. 5 shows how more frequent enzyme analyses, and especially CPK and LD/LDT give a better understanding of the course (7).

Case 5. A woman aged 69 with a history of hypertension and heart failure. She was admitted following pressurelike parasternal pains without radiation. On arrival at the CCU she was sweating and dyspnoeic. The ECG showed LBBB and the GOT and GPT values in the ordinary routine were difficult to use for measuring the extension of heart muscle necrosis. The curves of the CPK

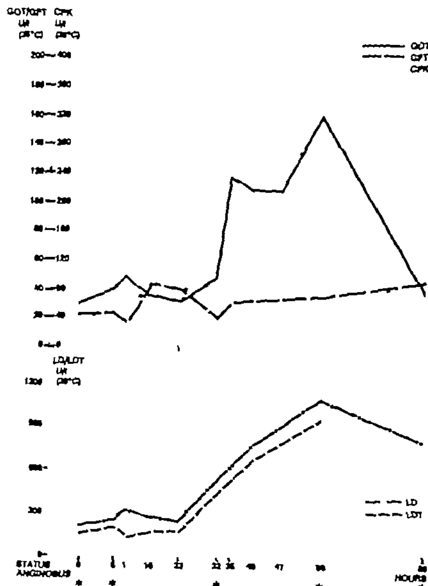


Fig. 4. A three-peaked curve in patient (case 4) with status anginosus culminating in an acute myocardial infarction. Symbols as in Fig. 1

and heart stable LDH clearly show however that the patient had an acute myocardial infarction.

With no other positive laboratory findings but serum enzymes supporting AMI the diagnosis is usually confirmed only if the values are clearly above the normal range. By using a more precise method and frequent analyses it is possible to ensure the diagnosis AMI as can be seen in Fig. 6.

Case 6. A 63-year-old man with history of possible myocardial infarction. On the day of admission he felt burning pain retrosternally of varying intensity. The ECG revealed no changes in the CCU nor was there any enzyme rise. A few days later he was readmitted to the CCU from an ordinary ward following the onset of similar pains. Now the enzyme analyses show a small myocardial infarction.

A still smaller myocardial lesion may result in no pathological laboratory findings but a typical enzyme pattern within normal GOT and LD values and maybe one or two CPK values in the upper borderline. Figs. 7 and 8 show two such patients, whose diagnoses could be confirmed only by careful and frequent enzyme analyses.

Case 7. A 52-year-old woman. She had angina pectoris for two years and was also found to have hyperlipaemia of type IV. Repeated chest pains during 15 hours before

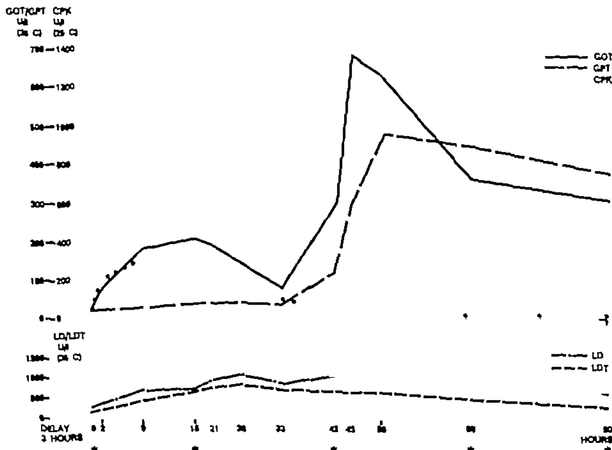


Fig. 5. Enzyme curves in a patient (case 5) with acute infarction complicated by a hepatic damage. Frequent determinations revealed curves of cardiac origin before the hepatic influence. Symbols as in Fig. 1.

admission. At admission continuous severe chest pain with radiation to the left arm and the back. The ECG showed localized ST elevation in CR_4. Routine analysis of the enzymes revealed GOT max. of 24 U/l. The curve, however, shows a pattern compatible with an acute myocardial infarction with a GOT max. of 30 U/l after an initial CPK rise and before a very moderate elevation of the heat-stable LD. This patient did not fulfil the generally accepted criteria of acute myocardial infarction but the diagnosis of intermediate coronary syndrome (ICS). A coronary angiography showed severe 3-vessel disease.

Case 8. A man aged 55 with cervical spondylosis and for the last year angina pectoris. At admission to the CCU the patient had retrosternal pain with radiation to the right arm, more intense at provocation with passive movements of his head. Fig. 8 shows the enzymes on this occasion and three weeks later when the patient was readmitted to the CCU with the same history. On the latter occasion there was a marked ST elevation in CR_4. Routine analysis shows a pattern compatible with myocardial necrosis. Diagnosis: intermediate coronary syndrome (ICS).

The patients of the ICS group differ in some clinical data from the other two diagnostic groups of patients (Table IV).

DISCUSSION

It is generally considered that histopathologically verified AMI patients nearly always have GOT elevations, depending on how often and by which method the analyses are performed (2, 3, 6, 8). These reports are based on autopsies and assume that all CCU patients with enzyme values below a certain limit do not have AMI. They are considered normal—without myocardial lesion.

Improved diagnosis of acute myocardial infarction thus simultaneously improves the diagnosis of non AMI conditions (12). In Table IV some clinical data in the three diagnostic groups are

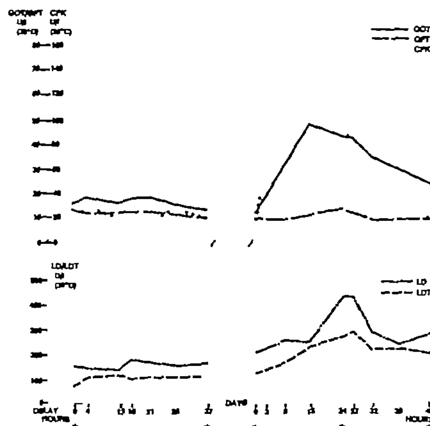


Fig. 6. T curves in patient (case 6) with prodromal angina followed by small infarction 4 days later. Symbols as in Fig. 1.

shown. All the patients in the ICS group had previous manifestations of ischaemic heart disease. The immediate prognosis was good, since there was no mortality during the stay in hospital.

Serum CPK determinations ha e been used in this study and seem to provide supporting information in most of the ICS patients, as can be seen from the Figures. As found by Crowley (4), however they are less discriminatory than GOT determinations in detecting myocardial infarction. Among the observation group patients 18% had pathological CPK alues, but in this group there are some patients with skeletal muscle damage and with epileptic attacks just before admission which may explain these findings.

Frequent enzyme analyses during the first day after admission to the CCU may thus result in an improved diagnosis. A careful observation of even small changes in the enzyme alues will give a better understanding of the patients in the borderline group between angina pectoris and acute myocardial infarction. The increased cost of more frequent determinations is low in comparison to the benefit of an improved diagnosis.

Table IV. *Some clinical data in the three groups of patients*

	AMI ( )	ICS (%)	OBS ( )
Male	75	47	51
Female	25	53	49
Previous hypertension	19	21	18
Previous heart failure	36	68	54
Diabetes mellitus	7	21	5
Previous myocardial infarction	37	58	39
Previous angina pectoris	50	95	64
Mortality during stay at hospital	22	0	7

ACKNOWLEDGEMENT

This study has been supported by grant from the Swedish National Association against Heart and Chest Diseases.

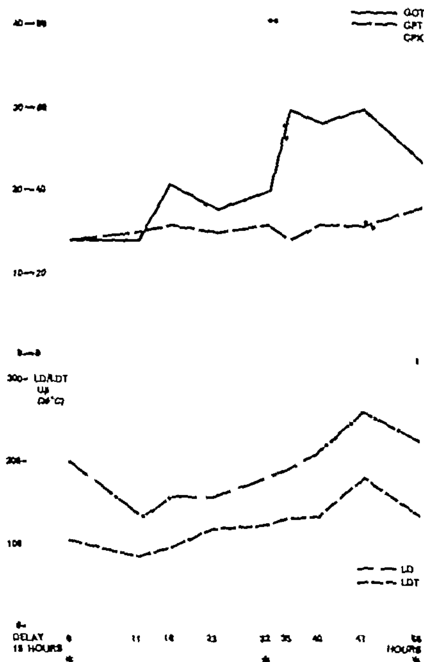


Fig. 7. A slight elevation within the normal values of GOT and CPK in a patient (case 7) with central chest pain for 15 hours. Intermediate coronary syndrome. Symbols as in Fig. 1

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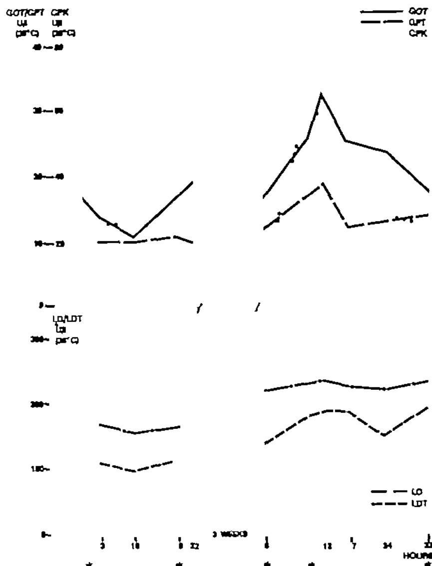


Fig. 8. T curves in patient (case 8) with two different periods of chest pain. The first curve represents an attack of angina pectoris and the latter of intermediate coronary syndrome. Symbols as in Fig. 1.

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Acta med. scand. Vol. 193 pp. 525–531 1973

LATENT CORONARY INSUFFICIENCY IN YOUNG ATHLETES

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Abstract. Staff and students at the Norwegian College of Physical Education and Sport, totalling 170 males and females of mean age 24 years, have been subjected to bicycle ergometer test with post-exercise ECG recordings. Sixteen athletes needed further evaluation for the following reasons: 1) ST depression of more than 1 mm. 2) Ventricular extrasystoles in the immediate post-exercise tracing. 3) A possibly incomplete RBBB. 4) Inadequate heart rate in the immediate post-exercise tracing. On reexamination with graded, submaximal bicycle ergometer test with in- and post-exercise ECG recordings, 8 persons were found to have normal response, 4 ECG changes of functional type, 2 ventricular extrasystoles, mainly as bigeminy which disappeared on high work loads, and 3 ECG changes of ischemic type. Selective coronary angiography was performed on the 3 athletes with latent coronary insufficiency and showed normal coronary arteriogram in 1 and hypoplasia of the circumflex coronary artery in 2. Problems of ECG of athletes and evaluation of the coronary arteriograms are discussed.

Latent coronary insufficiency may be defined as the presence of pathologic ECG changes of ischemic type either at rest or during or after exercise in an asymptomatic person. It is by now a well known clinical entity and reports on follow-up studies from 5–10 years have shown a great increase in morbidity and mortality of coronary heart disease in persons with latent coronary insufficiency (1, 4, 5, 22, 26, 27, 34).

In 1969 one of the students at the Norwegian College of Physical Education and Sport died suddenly during routine physical exercise in the swimming pool. The medical history had been uneventful until death, but on autopsy old and recent scars of myocardial infarction were disclosed with extensive coronary atheromatosis. As a result of this occurrence the staff and students at the college were subjected to an ECG exercise test. The result of this examination and, particularly the possible connection between latent coronary insufficiency and coronary artery hypoplasia as evaluated from selective coronary angiography are discussed in this paper.

MATERIAL AND METHODS

Staff and students at the Norwegian College of Physical Education and Sport in 1969 consisted of 67 females and 103 males, altogether 170 persons. Their age varied between 20 and 60 years (mean 24). All were subjected to 6-lead resting ECG including leads I, II, III, V and 3-lead in- and post-exercise ECG with the bipolar chest-head leads CH, ECG was recorded on 3-channel portable electrocardiograph (Cardiolux Epsilon 3 Elettronica Trentina AG, Italy). Exercise was done on mechanically braked bicycle ergometer (Monarch, Sweden) until heart rate (HR) of 170 to 180 was attained within 2–3 min. The in-exercise tracings were technically poor due to muscle disturbances, which pose greater problems in athletes than in normal persons. Therefore the immediate post-exercise tracing was used for evaluation. A second post-exercise tracing was recorded 5 min later.

Sixteen athletes needed further evaluation because of suspected abnormalities in the screening post-exercise ECG.

The examination was repeated with graded, submaximal exercise test on bicycle ergometer (Monarch, Sweden), starting with work load of 600 kpm/min for women and 900 kpm/min for men and increasing the work load by 300 kpm/min every 4th min until HR of 180 was attained. The ECG was monitored on 2 channel oscilloscope and recorded on 6-channel electrocardiograph (Elema Mingograph, 61 Sweden).

Selective coronary angiography was performed according to the Judkins technique (19) with the exception that cinetechnique was applied.

RESULTS

The resting HR was 71.0 (S.D. 16.2) and the HR immediately after exercise 150.0 (S.D. 13.0). Five minutes after exercise the HR had returned to

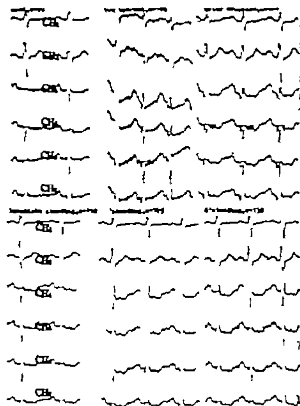


Fig. 1. Biphasic T waves and ST depression afte ex ercise, increasing during an orthostatic test (case 4).

84.7 (S.D. 12.2). Out of the total of 170 athletes 'ned, 16 turned out to ha e the following .lues in the ECG. 1) ST depression of than 1 mm of horizontal or slowly ascending type in any tracing—12 persons. 2) Ventricular extrasystoles in the immediate post-exercise trac ings— persons. 3) A possible incomplete RBBB in the immediate post-exercise tracing—1 person. 4) One person attained a HR of only 80 in the immediate post-exercise tracing.

On reexamination of the 16 persons the exercise test was found to be completely normal in 8 cases and we feel this to be due to improvement in the method used.

In . athletes ventricular extrasystoles, mainly as bigeminy appeared during exercise when the HR was between 120 and 150, but disappeared on further increase in HR and cardiac load. One of them had supraventricular extrasystoles immediately after work.

Three athletes had ECG changes consisting of ST depression of more than 1 mm and biphasic T waves on moderate exertion, which disappeared when cardiac load and HR were increased but returned within a few minutes after work. These abnormalities were considered to be functional and in part due to influence from the sympathetic nervous system (30). One female among them is also mentioned above as having ventricular extrasystoles. One person (case 4, female 24 y.) could not be tested properly because of a knee damage. She worked for a short time on a heavy work load and the in-exercise tracing showed no ST depression. The post-exercise ECG, however, showed ST depression and biphasic T waves which increased during an orthostatic test (Fig. 1). These were also classified as functional.

In two females and one male, pathologic tracings of ischemic type were found both during and after exercise (Figs. 2–4). Case 1 (female 23 y.) had a family history of diabetes and coronary heart disease. She had a diabetic oral glucose tolerance test. Case 2 (female, 25 y.) had a family history of myocardial infarction (3 relatives), whereas case 3 (male, 27 y.) had a normal family history. BP, protein-bound iodine, cholesterol and triglyceride values were normal in all three, and

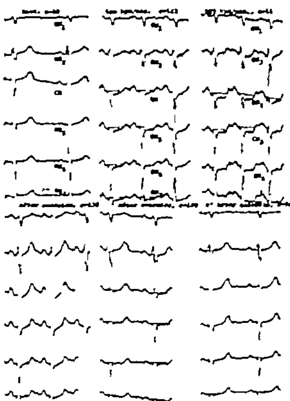


Fig. 2. Exercise ECG of case 1.

cases 2 and 3 had a normal oral glucose tolerance test. None were cigarette smokers.

Out of the total of 170 athletes, therefore the diagnosis of latent coronary insufficiency was established in three. These three volunteered for selective coronary angiography in view of the serious implications of the diagnosis. Selective coronary angiography revealed normal anatomy of the coronary arteries in case 1 and a hypoplasia of the circumflex branch of the left coronary artery with dominance of the anterior descending and right coronary artery in cases 2 and 3 as shown in Figs. 5 and 6. There was no evidence of atheromatosis or occlusive coronary artery disease.

DISCUSSION

In a group of 170 athletes examined with an ECG exercise test the incidence of latent coronary insufficiency was found to be 1.8%. This finding was surprising, as the incidence reported in young normals is lower (2).

The possibility that athletes might have re-

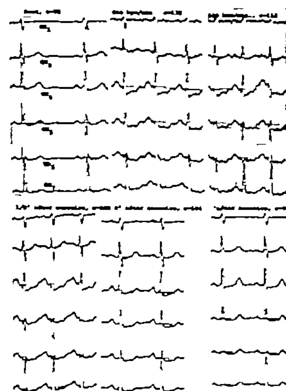
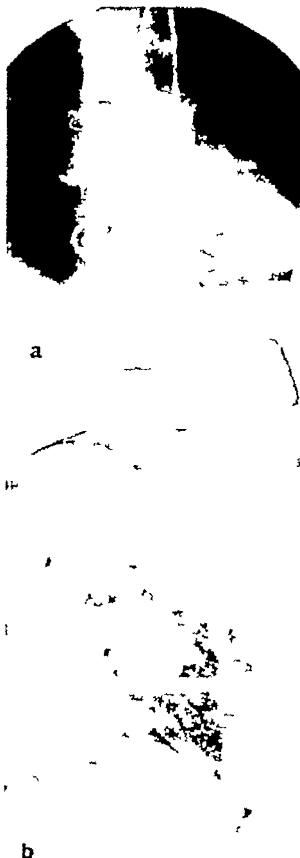


Fig. 3. Exercise ECG of case 2.



Fig. 4. Exercise ECG of case 3.

polarization changes simulating coronary insufficiency had to be considered. Most studies reported in the literature deal with changes found in the resting ECG of athletes. These changes consist of lower HR, longer PQ, QRS and QTc intervals, higher T waves in lead II, left axis deviation of the T wave, higher R and S amplitudes in the right and left precordial leads (10). Changes in the configuration of QRST in lead V_1, so-called notching and "shouldering" are more common in athletes than in untrained subjects (14). Repolarization changes, among which ST elevation and negative T waves, have been reported in champion cyclists during prolonged competitions (25). Recently Hanne-Paparo et al. (13) published the finding of T wave abnormalities in multiple leads in 7 top-ranking Israeli athletes. The abnormalities consisted of T wave inversions which in four disappeared immediately after a standardized exercise test (150 W performed for 3 min), but reappeared within 3 min after exercise in all but one. The authors suggest that a more strenuous exercise test might have abolished the



T wave inversions in the other three cases too. Their cases closely resemble four of our athletes whose ECG changes were classified as functional although they had ST depression as well. In this connection we should like to stress the importance of in- and post-exercise tracings. The exercise ECG should be subjected to a dynamic interpretation, and only ST T changes which progress as cardiac work increases qualify for the diagnosis of coronary insufficiency. In three athletes without clinical signs of coronary heart disease the exercise ECG revealed definite signs of myocardial ischemia and the changes could not be explained by strenuous athletic activity. The ECG diagnosis of myocardial ischemia has led to rejection from athletic participation (28). Selective coronary angiography as part of the diagnostic procedure in the study of latent coronary insufficiency has in the available literature only been described in a report on medical examination of aircraft personnel (8).

In one of the athletes (case 1) the coronary arteries were found to be normal. The paradox of normal coronary angiograms in patients with documented coronary heart disease has been reported by several authors (9, 21, 29). Dwyer et al. (6) have shown that the hemodynamic and clinical abnormalities in a group with angina and normal coronary arteries, compared to a group with documented coronary artery disease, were essentially the same, and lactate production has been demonstrated both during isoproterenol infusion (20) and during atrial pacing (24). Several explanations of this paradox have been offered, the most important being:

(a) Poor technical quality of the angiograms—this will hardly explain the majority of the cases. Moreover autopsy in some cases has verified the angiographic diagnosis of normal coronary arteries and also shown subendocardial infarctions (7) in some of these patients. (b) An abnormal hemoglobin-oxygen dissociation curve (7) in these patients, a finding which has not been verified by others (32). (c) Small vessel disease (16, 17). A family history of diabetes and an abnormal glucose

Fig. 3. Hypoplasia of the circumflex coronary artery in case 2. The right coronary artery (a) and the anterior descending artery (b) are the dominant arteries with wide inside diameters, while there is hypoplasia of the circumflex artery (arrow).





Fig. 6. Hypoplasia of the circumflex coronary artery in case 3. Left coronary artery seen in AP view (*a*) and in right anterior oblique view (*b*). Right coronary artery seen in AP view ( ). The anterior descending (large arrow), the obtuse marginal (small arrow) and the right coronary arteries are the dominant ones ith wide inside diameters, hile there is hypoplasia of he circumflex coronary artery (open arrow).

tolerance test are often found in patients with angina pectoris and normal coronary arteries, and studies of the retinal microcirculation have shown pathologic changes in many of these patients (33). Our case 1 presented a family history of coronary heart disease and diabetes on the mother side, and she had herself an abnormal glucose tolerance test. The remaining two athletes had hypoplasia of the circumflex branch of the left coronary artery.

The incidence of coronary artery hypoplasia has been studied in 3 400 autopsies by Berkheiser (3) and was found to be 1.4%. He measured the inside diameter of the main coronary arteries 1 cm peripheral to the orifice and found the mean diameter to be 8 mm in normals compared to 2 mm in the group with hypoplasia. Berkheiser does not comment upon the length of the hypoplastic arteries. The length of the arterial course, however aries considerably in all three main coronary arteries, especially in the circumflex artery (15). In our experience the distance between the orifice and point of termination will often be shorter in a hypoplastic artery whereas the remaining arteries may be dominant with wide inside diameters and an increase in length. We ha therefore used the following definition of coronary artery hypoplasia when an angiographic diagnosis is made:

1) The inside diameter 1 cm peripheral to the orifice should be 25% or less of that of the dominant arteries. 2) The distance between orifice and point of termination is mostly decreased in the hypoplastic artery. The normal length of the coronary arteries is based on the study of the anatomy of the coronary arteries in the monograph by James (15). 3) The length as well as the inside diameters of the remaining arteries are often increased.

In accordance with this definiti h pop

present in the circumflex coronary artery in two of the athletes with latent coronary insufficiency

Coronary artery hypoplasia without associated atheromatosis as the cause of myocardial infarction and/or angina pectoris has to our knowledge only been reported clinically by Grossman et al. (11 1) and later by one of us (31) Increased susceptibility to myocardial infarction and sudden death is found when coronary atheromatosis is present in small coronary arteries (34) or when coronary blood flow is compromised in one of the major vessels and the other is hypoplastic (3).

Coronary artery hypoplasia is a congenital anomaly and little is known of the prognosis. The combination of latent coronary insufficiency and coronary hypoplasia is of great interest and requires further observation, as the mortality and morbidity resulting from manifest coronary artery disease in persons with latent coronary insufficiency have been reported to be high.

Sudden death in association with physical exertion has been reported by James et al. (18) in two young men where autopsy revealed fibrosis in the region of the sinus node and changes in the sinus node artery and by McClellan and Jokl (23) who described seven cases with various congenital anomalies of the coronary arteries as the cause of sudden death. Coronary artery hypoplasia in combination with other anomalies was found in three of them. The role of physical exertion as the precipitating cause of sudden death in persons with coronary atheromatosis is, however not clear

The decision was made to let the three athletes with latent coronary insufficiency continue their athletic training and education. None of them were champions, but they wanted to teach athletics. We felt that physical training could be beneficial by possibly opening up collateral vessels. The athletes were told to avoid sudden very heavy exercise and concentrate on endurance training.

Two of them have been reexamined after one year with a routine clinical examination including a graded exercise test, and the ECG findings were unchanged.

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Acta med. scand. Vol. 193, pp. 533–536, 1973

THE NATURAL HISTORY OF INTERMEDIATE CORONARY SYNDROME

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Abstract. The prognosis in 132 patients with intermediate coronary syndrome (ICS) admitted to a coronary care unit has been investigated. All patients were monitored for at least 24 hours and they stayed in the hospital for at least 5 days. The observation period was 2 months. Sudden death occurred in 5 patients, fatal acute myocardial infarction (AMI) in 2 and non-fatal AMI in 15. Altogether 22 events (16.7%) occurred, 9 during the first 2 days, all except 3 during the first 3 weeks. The incidence of events has been examined with regard to previous coronary heart disease, sex, age, ECG changes and enzyme levels, but no significant differences were found. The status as regards angina of effort after 2 months in the 110 patients without events was unchanged in 79 patients and worse in 31 patients. Four patients had intractable angina. The results indicate that patients with ICS are liable to suffer from AMI within a short time. The risk declines gradually and reaches a fairly steady state during the second month. These patients should be kept under surveillance in hospital during the first days and in close contact with a hospital during the following month. The risk and benefit of coronary surgery in these patients should be weighed against the fatality incidence of infarction and of intractable angina mentioned above.

Prolonged chest pain suggestive of acute myocardial infarction (AMI) but unsupported by ECG and enzyme evidence, has been interpreted as a clinical syndrome intermediate between angina of effort and AMI, the intermediate coronary syndrome (ICS) (10). Other terms have also been used to describe this condition, the most usual being preinfarction syndrome (11), impending myocardial infarction (4) and acute coronary insufficiency (3, 15). The syndrome is common and therapeutically important. It accounts for 18% of admissions to our coronary care unit (CCU) and for 19% in the CCU of the Massachusetts General Hospital (3). The differentiation between ICS and small AMI as well as more extensive angina depends upon different and more or less arbitrarily chosen definitions. The incidences in other CCUs may therefore vary.

ICS as well as first and recurrent angina of effort are the most common and specific symptoms preceding AMI. Prospective studies have shown that these prodromata are seen in 60–70% of patients during the last 2 months prior to an AMI (7, 9). Information on how often these symptoms are not followed by heart attacks is, however, more uncertain (13). The natural history of this high-risk group is of great importance for the admission and discharge policy in our CCU. Patients liable to have an AMI within a short time interval can be kept under surveillance until the danger has passed. Fatal arrhythmic complications during the first minutes after an AMI could then be prevented.

The natural history of ICS is now important also since coronary bypass operation has recently become available for the management of this condition. The new therapy may be both effective and hazardous. The risk and benefit of this operation must be weighed against the natural course of the illness.

MATERIAL AND METHODS

The analysis is based on a consecutive series of 132 patients with ICS admitted to the CCU at Diakonissehusets Hospital, Oslo, from Jan. 1970 to April 1972. Only patients admitted within 24 hours after onset of symptoms were included. A 12-lead ECG, serum enzymes and other laboratory tests were taken immediately after admission and daily on the following 4 days.

A diagnosis of ICS was made if all the three following criteria were fulfilled.

1) A clinical history with typical chest pain (14) of more than 15 min duration.

2) Transient ST-T changes in the ECG in connection with the attack. In patients with verified preexisting coro-

Table I. *Incidence of death and non-fatal infarcts within 2 months in 132 patients with ICS*

Status on entry	No. of cases	Death: Sudden	Death: Fatal AMI	Non-fatal AMI	All events
No previous CHD	48	0	2	10	12
Angina of effort	45	2	0	3	5
Old infarction	39	3	0	2	5
All cases	132	5	2	15	22

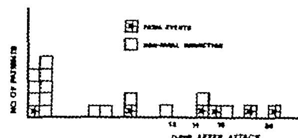


Fig. 1. Fatal events and non-fatal infarctions in 132 patients during the first 3 weeks after an attack of ICS.

nary heart disease (CHD) ST T changes were not required.

3) Absence of the ECG and laboratory signs of AMI mentioned below

All patients were minutely examined for preexisting CHD (angina of effort and old infarction) as well as for prodromal symptoms in the last 2 months prior to admission. The grouping of patients according to previous CHD was done without regard to prodromal symptoms.

A continuous ECG was monitored in all patients until at least 48 hours after onset of attack. All patients stayed in the hospital for at least 5 days, most of them 7-12 days. Antiarrhythmic and other treatment was given if needed. As soon as AMI was excluded, the patients were mobilized.

The patients were controlled clinically including ECG and laboratory tests after 2 months, or whenever there were episodes suggestive of AMI. A few patients were followed up by correspondence. The clinical course was estimated according to occurrence of

A. *Sudden death.* Death occurring within one hour of the onset of symptoms and without definite signs of AMI.

B. *Definite AMI.* Fatal or non-fatal, based on one of the following two criteria: 1) Very probable ECG changes (14), 2) Possible ECG changes (14) connected with SGOT > 50 U and SCPK > 120 U.

C. *Recurrent suspected or definite ICS.* New attacks of chest pain suggestive of AMI causing rehospitalization or continuous hospitalization for more than 21 days.

D. *New or aggravated angina of effort.* In patients without serious events the condition 2 months after admission was compared with the condition before the attack of ICS or before prodromata if present.

For comparison, patients with uncomplicated AMI admitted during 1970-71, and within 24 hours after the onset of the attack were observed in the same way as mentioned above. This group comprises 163 patients. Patients who had asystole, ventricular fibrillation or ventricular tachycardia during the first 48 hours were excluded, as well as patients who at 48 hours were hypotensive or in ventricular failure.

Table II. *Incidence of serious events in different groups of patients with ICS*

	No. of cases	Serious events[a]	Rate (%)
Sex			
Female	37	6	16.2
Male	95	16	16.8
Age			
< 60	46	8	17.4
60–69	55	9	16.4
≥ 70	31	5	16.1
ECG changes			
Transient T inversion > 1 mm	61	12	19.7
Transient T inversion ≤ 1 mm	29	4	13.8
Transient ST-T depression	15	3	20.0
Old infarction, LVH[b] and BBB[c]	27	3	11.1
Serum enzyme values			
GOT > 35 U or CPK > 75 U	42	6	14.3
GOT < 35 U and CPK < 75 U	90	16	17.8

[a] Sudden death, fatal and non-fatal AMI.
[b] Left ventricular hypertrophy.
[c] Bundle branch block.

RESULTS

The average patient age was 63 years (range 37–84). There were 37 females and 95 males. Forty-eight patients had no known preexisting CHD, 45 had prior angina pectoris and 39 had verified old infarction with or without angina of effort.

During the first 2 months sudden death occurred in 5 patients, 3 died in hospital and 2 at home. AMI occurred in 17 patients with 2 deaths, both in hospital (Table I). One patient had a silent AMI with significant ECG changes, the other patients were in hospital or were admitted to hospital in connection with their AMIs. Nine patients had their AMIs during the first 18–36 hours. Altogether 22 events (16.7%) occurred, all except 3 within 3 weeks (Fig. 1).

Table III. *Status 2 months after an attack of ICS in 110 patients who experienced no serious events*

Status on entry	No. of cases	Angina of effort: Present and worse	Present, but unchanged	Absent
No previous CHD	36	18		18
Angina of effort	40	10	30	0
Old infarction	34	3	29	2

Among 48 patients without preexisting CHD there were 12 AMIs with 2 deaths. Among 45 patients with angina of effort there were 3 AMIs and 2 sudden deaths. In the remaining 39 patients with old infarction 2 AMIs and 3 sudden deaths were seen (Table I). The incidence of fatal events was the same in the different groups. Non-fatal AMIs were seen more often in patients without preexisting CHD and the total incidence of events was therefore higher in this group. The difference was, however not significant. The incidences of events were also examined with regard to sex, age, different types of ECG changes and serum enzyme levels (Table II). No significant differences were found.

Among 163 patients with uncomplicated AMI 10 serious events (6.1%) occurred during the first 2 months. Four patients died suddenly 6 had a new AMI, 2 of which were fatal. All except 3 events occurred within 3 weeks.

Prodromata during the last 2 months prior to admission were seen in 75 patients (57%), more often in patients without previous CHD (76%) than with previous CHD (51%). No difference was found in the incidence of events between patients with and without prodromata.

During the following 2 months 110 patients (83 3%) had no serious events. Less serious attacks of chest pain were, however seen before as well as after admission. Altogether 64 patients (48 5%) had more attacks than the one leading to hospitalization. The incidence of attacks was highest on the last days before and the first days after admission. In most of the patients the attacks ceased during 7–10 days. Two patients, however had to stay in hospital for more than 3 weeks because of recurrent attacks, and 3 patients were rehospitalized for the same reason

The condition among the 110 patients who experienced no serious events following an attack of ICS was as follows (Table III). Eighteen of the 36 patients without previous CHD complained of angina of effort, while the other half had no symptoms. Among 40 patients who had preexisting angina of effort, 10 said it was worse, while the emaining 30 said it was unchanged. Among 34 patients with previous AMI, 3 had angina of effort which was worse, 29 angina which was unchanged and had no symptoms. Altogether the status was unchanged in 79 patients and worse in 31. Among the latter 4 patients had intractable angina.

DISCUSSION

During the last years attention has been focused on the identification of patients liable to have an AMI within a short time (13). Conditions with already established acute CHD i.e. AMI, ICS and first or recurrent angina of effort, are well known high-risk groups. The immediate prognosis in AMI depends upon complications and re infarction. In 188 patients with uncomplicated AMI who survived the first week 7% died of cardiac disease and 8 3% had definite or suspected AMI during the next 7 weeks. During the following 10 months 1 1% died and 4.2% had AMI (3). Among 163 patients with uncomplicated AMI in our CCU 2.4% died suddenly and 3 7% had definite AMI during the first 2 months. During the next 4 months 0 6% died suddenly and 1 8% had AMI. These and other studies (6) demonstrate that the risk even in uncomplicated AMI is high during the first weeks. The risk declines, however and reaches a fairly steady state after 1– months (5)

The immediate prognosis in ICS and following onset of angina of effort has earlier been investigated to assess the alue of anticoagulant treatment. In a study comprising 400 patients (1) the high incidence of AMI (4 %) within 3 months of onset of the ICS is stressed. Nothing is, however said about the time during the observation period when all these AMIs occurred. In a similar study comprising 150 patients with ICS (15) only 10 7% had AMI within 2 months. In two other studies, which included patients both with ICS and recent onset of angina, the incidence of AMI was 11 9% among 318 cases within 2 months (4) and 14% among 100 cases within

$1\,^1/_2$ months (1). The incidences of sudden death are not stated in these four studies. Most probably they have been included among AMIs. This would explain the high mortality rate (40–70%) found. Recently a new retrospective study of the clinical course of 100 patients admitted to a CCU with ICS has been published (3). Patients who had infarction on the two first days after hospitalization were excluded. During hospitalization and the first month after discharge 2 sudden deaths and 7 AMIs, one fatal, occurred. The results in the present study are in good accordance with four of the studies (1 3 4 15) mentioned above. Among 132 patients with ICS 17 (12.9%) had AMI and 5 (3.9%) died suddenly within 2 months. The course after this period has not been investigated, but most probably a fairly steady state is reached during the second month as shown in earlier studies (1 3).

The results show that the risk of developing an AMI is greatest during the first days following an attack of ICS. If the first 2 days are excluded from the observation period, the results can be compared with those found in patients with uncomplicated AMI. In the ICS group 13 (9.8%) serious events occurred and in the AMI group 10 (6.1%). In these two groups the same symptoms, the same sex and age distribution and the same incidence of prodromata were found. They reflect degrees of the same disease with probably the same risk of a coronary occlusion. In AMIs new infarctions during the first days will be more or less marked by the symptoms of the first infarction.

About half of the patients who were admitted with ICS had more than a single attack of chest pain. Most of the attacks were not serious and occurred during the first days. All these patients were in an unstable condition which ended in a fatal event in 7 cases and in non-fatal AMI in 15. In the remaining 110 patients the condition gradually stabilized or worse in 31 and unchanged in 79 compared to the condition before the attack or before the prodromata if present.

Earlier studies have shown that patients with ICS have a significantly lower incidence of arrhythmias than patients with AMI (3 8). Serious arrhythmias are seldom seen in patients with ICS (8).

The results indicate that these patients should be kept under surveillance in hospital during the first days, but not necessarily in a monitored bed. During the following month the patients should be in close contact with a hospital. The risk and benefit of a coronary surgery in these patients should be weighed against

1) the 5% fatality rate, in other studies 3–9% (1 3 4 15), seen during the first 2 months,

2) an incidence of non-fatal AMIs of about 6% during the first 2 days and another 5% during the following weeks,

3) the development of intractable angina seen in about 3% in the present study

4) the fact that in the present study 15% had had no symptoms and 66% had tractable angina 2 months after the attack.

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Acta med. scand. Vol. 193, pp. 537–54., 1973

PLASMA CORTICOSTEROID RESPONSE TO METYRAPONE

A Simplified Assessment of Pituitary-adrenal Function

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Abstract. An oral metyrapone test has been carried out in 25 patients without endocrine disease. Metyrapone was administered in divided doses over 24 hours. For the assessment of the response a method for the determination of plasma corticosteroids, including cortisol and 11-deoxycortisol, has been established. The method is based upon the competitive protein binding principle. The results have been compared with those obtained by measurement of urinary excretion of 17-hydroxycorticosteroids (17-OHCS). The average increase in plasma corticosteroids was about 100% from the morning before the start of metyrapone administration till the next morning. With normal pituitary and adrenal function an increase in plasma corticosteroids of at least 5 µg/100 ml can be expected, and the plasma level on the morning after metyrapone should be at least 19 µg/100 ml. The response in urinary 17-OHCS excretion was greater: an average increase of 200% was observed. It may therefore be expected that determination of 17-OHCS in 24-hour urine gives a better discrimination between the normals and those with reduced pituitary reserve than evaluation of the corticosteroid response from a plasma sample. The latter method is, however, easier to carry out, since it does not require urine collections, and can therefore be used in out-patients.

Under normal conditions at least three variables determine the pituitary-adrenocortical activity: a circadian rhythm, a feedback mechanism, and the condition of stress. It is widely accepted that these variables predominantly act through the hypothalamus, which controls production of corticotropin by secretion of corticotropin-releasing factor (CRF). The adrenocortical secretion of cortisol is closely related to the ACTH concentration in plasma (4, 17).

Since at present, methods for determination of ACTH are not generally available for clinical use, the secretion of ACTH is determined indirectly by measuring corticosteroids in plasma and/or urine before and after a provocation test. If the response is subnormal, pituitary insufficiency should not be diagnosed before primary adrenocortical insufficiency is ruled out by testing the adrenocortical response to exogenous corticotropin (10).

At present four dynamic tests (see below) are in use to diagnose adrenocortical insufficiency secondary to hypothalamic or pituitary disease. Although the mechanisms are different and partly unknown, all these tests act by increasing the secretion of corticotropin.

Although both the pyrogen test (2, 8) and the lysin-vasopressin test (6, 11) have their advocates, they have been abandoned by others because of unpleasant and sometimes dangerous side-effects (22).

The insulin-hypoglycemia test is preferred by many authors (5, 7, 12), who regard it as safe, sensitive and reliable. An increase in plasma cortisol during hypoglycemia is an indication that the patient will respond adequately to stress. Simultaneously the pituitary capacity for growth hormone secretion can be assessed. The test may fail if the degree of hypoglycemia is inadequate. Hypoglycemia may, however, be dangerous in patients with hypopituitarism or coronary heart disease. In our hands the insulin-hypoglycemia test has been disappointing. In spite of adequate hypoglycemia, often accompanied by subjective symptoms, several of our normal control patients have shown no or only insignificant increase in plasma cortisol.

Like the other tests the metyrapone test (13) has both supporters and critics (19). One objection has been that metyrapone tests the feedback system only and tells nothing of the ability to

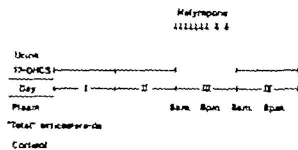


Fig. 1. Metyrapone was administered orally on day III. Starting at 8 a.m. 250 mg was given 7 times, each second hour, then 750 mg at 12 p.m. and at 4 a.m. (total 3 250 mg). Urine was collected for 24 h from 8 a.m. on days I, II and IV and was analysed for 17-OHCS. Plasma was drawn for determination of cortisol and "total" corticosteroids at 8 a.m. and at 8 p.m. on days III and IV.

respond to stress. In addition the absorption of metyrapone, when administered orally, is variable (19). The test, as it is usually done, is time-consuming, and accurate urine collection is necessary for three to four days.

Metyrapone inhibits selectively the 11β-hydroxylase in the adrenals and causes a decline in the production and in the circulating level of cortisol. This means a lowering of the negative feedback to the pituitary resulting in increased plasma concentration of ACTH (20). The adrenal corticosteroid production is thereby stimulated.

Until recently we have estimated the metyrapone response by measuring the urinary excretion of 17-hydroxycorticosteroids (17-OHCS) and tetrahydro-11-deoxycortisol (tetrahydro-S), a metabolite of 11-deoxycortisol, the last product before the metyrapone block.

The purpose of the present investigation has been to simplify the metyrapone test by adopting plasma methods for estimation of the response.

MATERIAL AND METHODS

A group of 25 patients has been examined, 15 women, mean age 48 years (14–79 years), and 10 men, mean age 61 years (19–76 years). The patients were hospitalized for different diseases, none had signs of endocrine disease, heart incompensation, renal or hepatic disorder.

Metyrapone (Metopiron®, Ciba) was given orally and the test was carried out on four days as shown in Fig. 1.

Urinary 17-OHCS was determined as described by Metcalf (13).

Two methods were used for the determination of plasma corticosteroids. A fluorometric method described by Mattingly (14) with the modifications introduced by de Moor and Steeno (3) was used to determine 11-hydroxycorticosteroids, designated cortisol in this paper. The other method was a competitive protein-binding method based upon the principle described by Murphy et al. (16). The measurement obtained with this method is called "total" corticosteroids, comprising steroids with high binding affinity to the cortisol-binding globulin transcortin.

The procedure is as follows: 0.1 ml of plasma is added to 1 ml of water and extracted once with 15 ml dichloromethane, and the extract is washed with 1 ml of water. An aliquot of 1 ml is taken out, evaporated to dryness, and assayed for the contents of corticosteroids, using late pregnancy plasma as the source of binding protein and [1,2-^{3}H] corticosterone of high specific activity (50 Ci/mmole) as the labelled steroid, as described by Johansson (9). The pregnancy plasma is diluted 1/500 and 1 ml of this dilution, containing about 60 pg and 18 000 D.P.M. [1,2-^{3}H] corticosterone, is added to the residue of the plasma extract. Standard curves are obtained by substituting the plasma extract with known amounts of cortisol. Incubation is carried out at 40°C for 5 min followed by 10 min in ice water.

The separation of the protein-bound radioactivity from the free radioactive steroid is accomplished with about 80 mg florisil under careful mixing for 30 sec. The florisil is allowed to settle while the samples are kept in ice water for 40 sec.

Five hundred μl of the supernatant is transferred to a counting vial, 10 ml of scintillation fluid (InstaGel, Packard) is added, and the radioactivity measured in a liquid scintillation spectrometer for 20 min (sufficient to obtain a precision of 2%).

Standard curves are constructed (Fig. 2) and the samples are calculated using the following formula.

$$\mu g \text{ corticosteroids}/100 \text{ ml plasma} = N \cdot \frac{100}{0.2} \cdot \frac{15}{1} \cdot \frac{1}{1\,000}$$

where N represents the amount of corticosteroids, expressed in ng, observed with the standard curve, 0.2 represents the volume of plasma extracted, and 15 refers to the fraction of the extract which is submitted to the binding assay.

Specificity. All steroids competing for the binding sites on the protein will be included in this measurement. In addition to cortisol, these steroids are primarily corticosterone, 11-deoxycortisol, progesterone and 17-hydroxyprogesterone. The relative activity of progesterone in the assay is 1.20 and of 11-deoxycortisol 1.16 compared to cortisol (10). Progesterone will therefore contribute significantly to the measurement during pregnancy and in the middle of the luteal phase of the menstrual cycle, at which time the plasma level of progesterone may go up to 1.2 μg/100 ml (21). This means that about 10% of the value observed for plasma corticosteroids in a normal woman one morning in the middle of her luteal phase is caused by progesterone.

The fact that cortisol, corticosterone and 11-deoxycortisol have very similar binding characteristics implies that the method measures the total amount of corticosteroids present.

Precision. The coefficient of variation between duplicate

determinations was found to be 7.8% for values between 10 and 46 μg/100 ml.

Accuracy was tested by calculating the recovery of cortisol added to plasma with known concentrations. The average recovery was 91.5% ± 6.7 (S.D.), which corresponds nicely to the recovery of the extraction procedure.

RESULTS

The individual response to metyrapone in 21 of the tested patients can be seen in Fig. 3. The most consistent response is the increased plasma level of "total" corticosteroids at 8 a.m. on day IV. The mean values at 8 a.m. before and the day after stimulation in the 25 patients are presented in Fig. 4.

A 30% reduction in plasma cortisol was observed, whereas the "total" corticosteroids increased by more than 100%.

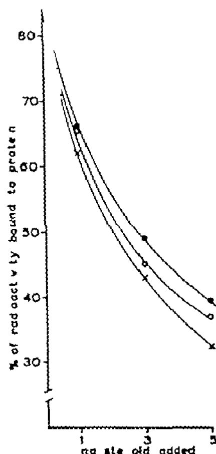


Fig. 2. Standard curves for cortisol ●—● 11-deoxycortisol ○—○ and progesterone ×—×.

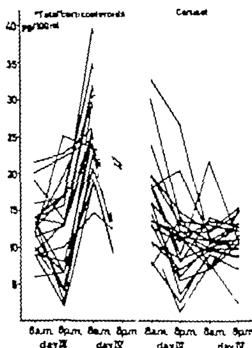


Fig. 3. "Total" corticosteroids and cortisol in plasma before (8 a.m. day III), during and after oral metyrapone administration in 21 control patients (10 men and 11 women, mean age 54 years).

The mean urinary excretion of 17-OHCS before and after metyrapone is presented in Fig. 5 where it can be seen that the excretion increased by more than 200%. A summary of all results is presented in Table I.

DISCUSSION

The normal response to metyrapone is an increased adrenocortical function, which may be reflected in an increased urinary excretion of 17-hydrocorticosteroids or increased plasma levels of corticosteroids. An increased plasma level of 11-deoxycortisol which has been used as a parameter for metyrapone response (18, 20) may however not necessarily reflect an increased adrenocortical function. Under certain autonomic conditions, like the ectopic ACTH syndrome or an adrenal adenoma, metyrapone will lead to reduced cortisol production and correspondingly increased 11-deoxycortisol production, whereas the total corticosteroid output remains unchanged. Therefore the very impressive response in plasma 11-de-

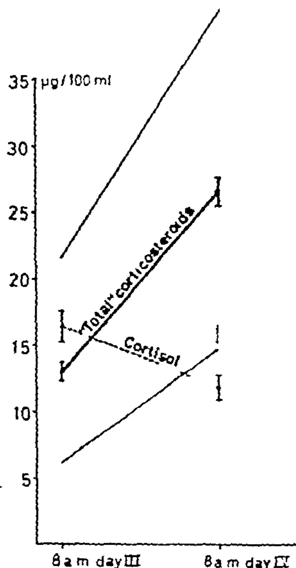


Fig. 4. Mean plasma levels of "total" corticosteroids and cortisol before and after oral metyrapone administration in 25 control patients (10 men and 15 women, mean age 53 years). Range bars denote S.E.M. For "total" corticosteroids the range of the observations is also presented (shaded area).

oxycortisol or urinary excretion of tetrahydro-11-deoxycortisol is not under all conditions a more adequate way of expressing the response to metyrapone.

On theoretical grounds a similar argument may be raised in patients with limited pituitary ACTH reserve. There may be enough ACTH to maintain a normal cortisol level in the plasma in the morning. Following metyrapone administration 11β-hydroxylation is blocked, the adrenals excrete 11-deoxycortisol, which leads to increased plasma level of this compound, but as there is no ACTH reserve, the adrenal cortex cannot increase its total steroid output. Also in such patients the increased plasma level of 11-deoxycortisol does not reflect increased adrenocortical activity.

Since the main objective of the metyrapone test is to test the pituitary function, it is important that the parameter used for the evaluation correctly reflects the pituitary function. From what has been said both urinary excretion of 17-OHCS and "total" corticosteroids in plasma will do that, presuming that the adrenal function is normal.

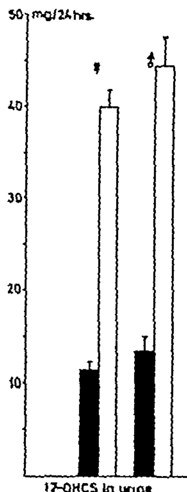


Fig. 5. Effect of oral metyrapone administration on the urinary excretion of 17-OHCS in control patients (7 men, mean age 59 years, and 11 women, mean age 45 years). Closed columns represent the mean 24-h excretion before metyrapone. Open columns represent the mean 24-h excretion on the day after metyrapone administration. S.E.M. is indicated by range bars.

Table I. *Effect of oral metyrapone administration (day III) on plasma corticosteroids and urinary 17-OHCS excretion*

The increment in plasma corticosteroids is the increase from the basal value (at 8 a.m. on day III) to the poststimulatory value (at 8 a.m. on day IV). The increment in urinary 17-OHCS is the difference between the excretion on day IV and the average of the excretion on day I and II

		Basal values			Increments			Poststimulatory values		
		Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range
"Total" corticosteroids in plasma (µg/100 ml)	25	13.0	3.6	6.0–21.5	13.5	5.0	4.5–26.0	26.5	5.7	14.5–40.0
Urinary 17-OHCS (mg/24 h)										
♂	7	13.4	4.1	8.0–19.8	29.0	6.5	23.[illegible]–37.3	42.4	7.8	30.7–53.8
♀	11	11.4	2.9	6.8–15.9	28.5	5.8	17.6–34.6	39.9	6.1	28.3–46.1

The increment in plasma "total" corticosteroids ranged from 4.5 to 26.0 µg/100 ml, the peak value after stimulation ranged from 14.5 to 40.0 µg/100 ml. The increment was below 10 µg/100 ml in only five patients, below 5 µg/100 ml in two. The peak value after stimulation was in only one patient below 19 µg/100 ml.

At present it is difficult to establish the lower limit of normal response to metyrapone. Until further experience is gained, we shall regard an increment in "total" corticosteroids in plasma of 5 µg/100 ml and a morning value after metyrapone not less than 19 µg/100 ml as the lower limit for normal response.

All 25 subjects tested fulfilled at least one of these criteria, 22 fulfilled both. In the three subjects with a "subnormal" response in plasma corticosteroids, two had clearly not adrenal insufficiency. Their plasma level of "total" corticosteroids were high before stimulation, so although the levels increased only 4.5 µg/100 ml after stimulation, the plasma levels rose to near the mean level for the whole group, 24.5 and 26.0 µg/100 ml, respectively. In the third subject the plasma corticosteroids rose from 9.0 to 14.5 µg/100 ml, the urinary 17-OHCS excretion rose, however, from 10.4 to 45.8 mg/24 h.

It appears that inclusion both of the increment and of the poststimulatory value (peak value) in the evaluation of the response will give a more complete impression of the pituitary-adrenal function than just estimating one of them. A low increment of corticosteroids in blood or in urine will be less warning when the rise is from a high than from a low basal level.

Metyrapone induced an increase in urinary 17-OHCS that was comparable to that found by Landon et al. (12). There is, however, considerable uncertainty about the criteria for the lower limit of normal response. Our results indicate a normal response to metyrapone to be an increment in urinary 17-OHCS of at least 15 mg/24 h or the excretion on day IV to be at least 25 mg/24 h.

The urinary excretion of 17-OHCS increased relatively more after metyrapone stimulation than did the concentration of "total" plasma corticosteroids. The reason for this apparent discrepancy is probably related to the fact that a plasma sample reflects a momentary situation, whereas the urinary excretion represents the whole day's function. It may therefore be expected that the discrimination between the normals and those with reduced pituitary function will be better when measuring the urinary excretion of 17-OHCS rather than the "total" plasma corticosteroids.

From the foregoing it follows that the increased ACTH activity after metyrapone is reflected nearly as well by the plasma corticosteroids as by the urinary 17-OHCS excretion, and plasma determination may therefore be used in a simplified screening test. The main advantage is that urine collection is not necessary. Plasma is drawn for determination of "total" corticosteroids at 8 a.m. Metyrapone is then administered as described, and the response is measured in a plasma sam[illegible] at 8 a.m. the next morning.

The test, which is carried out in 24 h [illegible] also be applied to out-pa[illegible] if they can [illegible]

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under strict observation and it can be assured that metyrapone is taken 2-hourly. This seems to be essential for adequate suppression of plasma cortisol (1, 19).

In most normals the metyrapone response will be well above the proposed criteria for the lower normal response. If the response is subnormal, the test should be repeated, also including determination of urinary 17-OHCS excretion. Before diagnosing pituitary insufficiency it must, however, be assured that the adrenals are able to respond to corticotropin.

On stronger suspicion of hypopituitarism we find it more adequate to include both the urinary and the plasma method, without first carrying out the simplified test.

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Acta med. scand. Vol. 193 pp. 543–546, 1973

BLOOD COAGULATION, FIBRINOLYSIS AND PLATELET FUNCTION IN WOMEN AGED 38, 46, 50, 54 AND 60

The Study of Women in Gothenburg 1968–1969

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Abstract. Blood coagulation, fibrinolysis and platelet function have been studied in a female population of 404 subjects in the age strata 38, 46, 50, 54 and 60 years. There was a significant increase in fibrinogen with increasing age. The population was also subdivided according to menopause. The results indicate that the menopause has some influence on the fibrinogen concentration. Partial thromboplastin time (PTT) in silicone tubes varied significantly with age. The curve was two-humped. This is probably the result of two opposing effects on the clotting time: the fibrinogen concentration which increases with increasing age and tends to prolong the clotting times, and an unknown factor which tends to shorten the clotting times with increasing age. This non-identified factor has probably something do to with surface activation of plasma.

Thrombotic disease is closely correlated to coronary heart disease, and changes in blood coagulation and fibrinolysis may be one of the explanations of the difference in the incidence of coronary heart disease which is seen between different groups of subjects. Coronary heart disease in women is more common after than before the menopause. It is therefore of interest to compare variables of blood coagulation and fibrinolysis in premenopausal and postmenopausal women. The purpose of this paper is to describe the influence of age and menopause on blood coagulation, fibrinolysis and platelet function in women in the ages around the menopause.

STUDY GROUPS

The present work is part of a comprehensive population study of women in Gothenburg, Sweden, carried out during the years 1968–69. The study groups were random samples of women in the age strata 38, 46, 50, 54 and 60 living in the city of Gothenburg and selected by the Revenue Office. A detailed description of the method of selection and the participation rate is presented elsewhere (1).

Blood coagulation and fibrinolysis were carried out on subsamples consisting of all women in Gothenburg born on the 6th day of every month of the year in 1908, 1914, 1918, 1922 and 1930, respectively. The numbers of subjects and participation rates in these subsamples are given in Table I.

Menstruating women were examined approximately 16 days after their menses. There was no difference between the subsamples in this respect. The examinations of the women from the different age groups were equally distributed throughout the months of the year. Thus the effect of seasonal variations cannot have had any influence on the results.

Platelet adhesiveness was determined only in those born in 1908 and 1930.

METHODS

Sampling of blood

The women arrived at the hospital at 15-minute intervals between 7.00 a.m. and 9.45 a.m. after overnight fast. Blood was collected from an antecubital vein with a minimum of stasis, using a 2 mm disposable steel needle with a 60 cm plastic tube attached. The first few ml were discharged and the blood was then collected directly into plastic tubes and mixed with 1:10 volume of 0.1 M citrate. The tube for fibrinogen determination also contained lysinethylester to prevent fibrinogenolysis. Approximately 5 ml of the citrated blood was left at room temperature for 1 hour and then used for determination of platelet adhesiveness. The rest of the blood was immediately centrifuged at 1 700 g for 20 min at room temperature. Plasma was collected with a silicone-treated pipette and placed in a silicone-treated glass tube. All the tests except the plasminogen determination were carried out immediately. The plasma samples were frozen and kept at −30°C. Plasminogen determinations were carried out once a week on the frozen samples.

Table I *Participation rate in the subsamples used for the studies of blood coagulation and fibrinolysis*

Age (y)	Called for examination (n)	Examined (n)	Participation rate (%)
38	81	66	81.4
46	110	93	84.5
50	93	83	89.2
54	97	83	85.5
60	97	77	79.3
Total	478	402	84.1

Coagulation and fibrinolysis

A detailed description of the methods used for the study of blood coagulation and fibrinolysis has been given elsewhere (3). The variables studied and presented in the present paper are: recalcification time of citrated plasma in silicone-treated tubes, partial thromboplastin time (PTT) both in silicone-treated tubes and in glass tubes, factor II-VII-X activity determined by the original PP method, factor VIII, fibrinogen, fibrinolytic activity in an euglobulin precipitate determined on fibrin plate, and plasminogen.

Platelet adhesiveness

Platelet adhesiveness was determined in citrated blood according to Hellem method (2).

Statistical methods

Conventional statistical methods were used for calculation of mean values and standard deviations. Significances of differences between groups were studied by means of Student *t*-test or analysis of variance. The difference was considered statistically significant for $p<0.05$. The data were analysed in computer (IBM 360 65). Mrs Gullbritt Palm has been consultant statistician and responsible for the computer analysis.

RESULTS

The age distribution is presented in Table II. Fibrinogen was found to increase continuously with increasing age. Analysis of variance showed the increase to be highly significant. PTT in silicone-treated tubes varied significantly with age. Recalcification time of citrated plasma in silicone tubes varied in a similar way. The variation was, however not statistically significant. PTT in glass tubes—the third variable measuring the total clotting potential of plasma—did not vary. Fig. 1 illustrates the increase in fibrinogen and the variation in PTT. It also shows that factor VIII varied in the opposite direction to PTT although the variation was not significant.

In Table III each group has been divided into subgroups of premenopausal and postmenopausal women. The total number in each age group is reduced compared to Table II, as 13 ovarectomized women, 23 women on whom hysterectomy had been carried out and 24 women with missing data concerning the menopause were excluded.

Table II *Age distribution in the study groups (F-values from analysis of variance)*

	38 y (n=66)	46 y (n=93)	50 y (n=83)	54 y (n=83)	60 y (n=77)	F	Significance
Recalcification time, silicone tubes (sec)	412±101	405±105	423±100	420±107	388±78	1.62	—
PTT (sec) Silicone tubes	230±42	210±48	219±44	227±46	204±34	4.88	$p<0.01$
Glass tubes	57±9	57±8	57±8	56±7	57±7	0.22	—
Factor II-VII-X (%)	93±15	95±18	97±15	98±14	93±17	1.66	—
Factor VIII (%)	134±62	140±65	134±66	131±61	143±56	0.52	—
Fibrinogen (mg %)	287±70	290±63	316±78	320±71	361±130	9.55	$p<0.01$
Spontaneous fibrinolytic activity (mm²)	117±26	119±22	115±20	122±27	112±20	2.01	—
Plasminogen (U/ml)	17.5±4.1	17.4±3.3	17.3±3.2	17.4±3.2	17.0±2.7	0.27	—
Platelet adhesiveness (%)	56±11				58±12	0.59	—

in silicone tubes, on the one hand, and fibrinogen on the other. As fibrinogen increases with increasing age, one should also expect the plasma clotting times to be prolonged with increasing age if no other factors had any effect. An explanation of the two-humped curve for PTT in Fig. 1 might be that the effect of the increasing fibrinogen concentration is counteracted by other factors which tend to shorten the clotting times. This would explain why the clotting times in premenopausal women aged 46 are shorter than in those aged 38. In women aged 50 and 54 the prolonging effect of the increased fibrinogen concentration might be balanced by factors giving shorter clotting times. In the oldest women the influence of the clot promoting factors might be strong enough to neutralize the effect of the increased fibrinogen concentration and finally result in a definite shortening of the clotting times.

Factor VIII activity has been shown to be negatively correlated to plasma clotting time and PTT in silicone tubes in men (3). In Fig. 1 it is shown that factor VIII activity tends to vary in the opposite direction to the clotting times, suggesting some connection between these variables. However the variation of factor VIII in the present series is not significant. There is no variation of PTT when determined in glass tubes. The only difference between the two PTT tests is that the degree of surface activation is high and standardized when the test is carried out in glass tubes. Variations in factor VIII activity should have influenced all three plasma clotting tests. It therefore seems probable that the clot promoting activity which gives shorter clotting times in plasma in the oldest women is different from factor VIII. With the design of the present study the factor(s) responsible for this activity could not be identified.

However the shorter clotting times were only demonstrable in the two tests which were carried out in silicone tubes and not in the test carried out in glass tubes (in which the in vitro surface activation is high). This fact indicates that the clot promoting activity may have been caused by in vivo surface activation. In comparing male patients who had sustained a myocardial infarction with a control group representative of the male population, we observed a similar result, i.e. a shorter recalcified plasma clotting time and a shorter PTT were observed in the postinfarction group compared to the control group when the tests were carried out in silicone tubes but not when carried out in glass tubes (4). It is tempting to believe that the older women and the postinfarction patients had a more extensive atherosclerosis than the control groups, and that the high degree of surface activation was caused by the rough surface of the vessels.

ACKNOWLEDGEMENT

Supported by grants B70-19X-2702-02B and B71-19X-2702-03 from the Swedish Medical Research Council.

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Acta med. scand. Vol. 193 pp. 547–550, 1973

A WITHIN-PATIENT COMPARISON OF ALPRENOLOL AND PROPRANOLOL IN HYPERTENSION

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Abstract. In an individually titrated dose cross-over trial of propranolol and alprenolol in 18 hypertensive patients both drugs were useful as antihypertensive agents although propranolol in the used dosages was found to be more effective and caused significantly greater reduction of arterial pressure both in the supine position (systolic $p<0.05$, diastolic $p<0.01$) and in the erect position (diastolic $p<0.0025$). Propranolol reduced the heart rate significantly more than alprenolol.

Since the first report on the usefulness of propranolol in lowering elevated arterial pressure (4) several authors have reported on adrenergic β-receptor blockers used as antihypertensive agents (3, 5, 7, 8). The treatment policy at our Hypertension Out-patient Clinic was at the time of this study to use propranolol or alprenolol as the first drug of choice for patients with previously untreated hypertension if there were no contraindications such as bronchial asthma or heart decompensation. The decision whether to give propranolol or alprenolol to the individual patient was based upon the date of birth. Patients born on even dates were given alprenolol and patients born on uneven dates received propranolol. We were interested to see which drug was the most potent hypertensive agent and, as no earlier comparison had been made, this study was started.

MATERIAL AND METHODS

From the above mentioned pool of patients treated with either propranolol or alprenolol for their hypertension the patients for the present study were recruited. There were 15 men and 3 women, whose average age was 47 years (range 28–64). All patients had essential hypertension. All had benign hypertension, nine of them in stage 1, eight in stage 2 and one in stage 3 as classified in accordance with the W.H.O. criteria (Table I).

Eleven patients were treated with propranolol initially and then changed over to the corresponding dosage of alprenolol, and seven patients started treatment with alprenolol and were then switched over to propranolol. In order to compensate for possible differences due to the fact that the same number of patients were not started on each drug, a double cross-over was undertaken in eight patients, six of them starting on propranolol and two on alprenolol.

Each patient had a run-in period on the starting drug of at least 2 months before the trial started. During this time the daily dosage necessary to obtain control of the BP was determined. After another 9 weeks on a fixed dosage of the first drug the treatment was then changed to the other drug for another 9 weeks. The dosage of the second drug was determined on a fixed ratio of 0.4, meaning that 40 mg propranolol was given instead of 100 mg alprenolol or vice versa. The 0.4 ratio was chosen instead of the more natural 0.5 ratio (which is the ratio closest to the difference of adrenergic β-receptor blocking capability between the two drugs when administered orally (9)), because with the 0.4 ratio it was possible to keep the daily number of tablets unchanged irrespective of whether the patient was treated with propranolol or alprenolol, as only 40 mg tablets of propranolol and 100 mg tablets of alprenolol were used in the study. The advantage of this was considered to be more important than to obtain an exact dose correspondence in regard to the β-blocking effect. The tablets used were supplied by the respective manufacturers and were of the usual commercially available composition for each brand.

Throughout the study all BPs were measured by one nurse. BP was measured in both arms, both in the supine position after 5 min rest and in the erect position after standing for 1 min, although only the pressures measured in the right arm have been used. The pulse rate was measured in the resting supine position.

The patients were seen in the Hypertension Out-patient Clinic at 3-week intervals. At each visit the following laboratory tests were performed: serum uric acid,

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Table I. Sex, age, duration of hypertension, eyeground changes according to Keith–Wagener–Barker, hypertensive stage according to the W.H.O. criteria, dose of β-blockers and other drugs in the 18 patients studied

A = alprenolol, P = propranolol, H = hydralazine, D = diuretics

	Sex	Age (y.)	Duration (mo.)	Fundi	W.H.O. stage	Dosage (mg) A	Dosage (mg) P	Other drugs
Group 1 (A→P)	♂	45	12	II	2	800	320	H+D
	♂	41	6	II	1	400	160	—
	♀	64	180	II	1	200	80	D
	♂	61	8	II	2	200	80	—
	♂	47	84	II	2	300	120	H+D
Group 2 (P→A)	♂	52	12	II	1	800	320	H+D
	♀	28	18	I	1	600	240	—
	♂	49	60	II	2	800	320	—
	♂	39	12	II	2	800	320	H
	♂	39	60	I	2	800	320	—
Group 3 (A→P→A)	♂	50	12	I	1	800	320	H
	♂	62	120	II	1	800	320	H+D
Group 4 (P→A→P)	♀	29	12	0	1	600	240	—
	♂	48	6	I	1	400	160	—
	♂	48	12	—	2	600	240	H
	♂	53	72	III	3	1 200	480	H+D
	♂	50	12	II	2	800	320	H
	♂	33	6	I	1	200	80	—

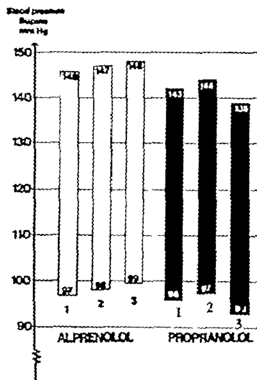


Fig. 1. Average systolic and diastolic BP in the supine position at the three determinations on each drug.

serum bilirubin, serum alkaline phosphatases, SGOT and SGPT.

The average dosage of propranolol was 247 mg daily (range 80–480) and of alprenolol 617 mg daily (range 200–1 200). Nine patients received additional treatment with hydralazine and six patients took diuretics. The dosage of this additional therapy was kept unchanged throughout the study.

The average systolic and diastolic BP before the run-in period started was almost identical in the two study groups; 183/114 for the 11 patients starting on propranolol and 182/113 for the 7 starting on alprenolol.

Statistical methods

The mean of the three BP recordings on each drug was compared statistically by using the Student's *t*-test for the means of differences between paired observations. In this way each patient acted as his own control throughout the study. The pulse rates and the results of the laboratory tests were compared in the same way.

RESULTS

Effects on arterial BP

The average systolic and diastolic BP recorded at the three determinations on each drug are seen in Figs. 1 and 2. Statistical analysis of the data from the third determination showed a significantly lower diastolic BP on propranolol treatment ($p < 0.01$ in the supine and $p < 0.0025$ in the standing position) and also lower systolic BP on

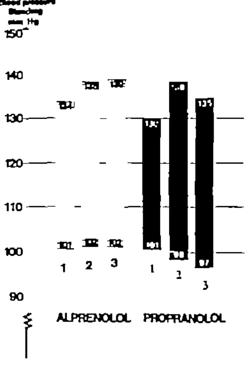


Fig. 2. A erage systolic and diastolic BP in the standing position at the three determinations on each drug.

propranolol in the supine position $p < 0.05$ (Fig. 3).

Effects on heart rate

Propranolol also reduced heart rate by 8 beats/min more than alprenolol, which was statistically significant ($p < 0.0005$).

Effects on laboratory data

No statistically significant differences were seen in Hb, bilirubin, SGOT SGPT alkaline phosphatases or uric acid. One patient had a mild proteinuria which was unchanged throughout the study

Side-effects

One patient had to discontinue treatment during the propranolol period because of bradycardia. The lowest pulse rate recorded was 48 beats min and, as the patient was distressed by this, treatment was stopped. Four patients had to discontinue treatment while on alprenolol. All four had previously tolerated propranolol. One of these patients developed widespread urticaria on the second day of alprenolol treatment. Another patient, a man aged 44 complained of breathlessness, claiming he was able to walk only 10 m. Because of this he spontaneously discontinued alprenolol treatment. Two other patients on alprenolol were removed from the study because their diastolic BP rose above 115 which was a prefixed limit for adherence to the study None of the five patients who discontinued treatment are included in the group of 18 patients who completed the course. Other side-effects during propranolol treatment were tiredness, which was mentioned by two patients, while two patients complained of headaches and dizziness during alprenolol treatment.

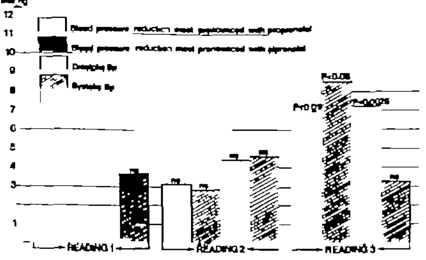


Fig. 3. Mean difference in BP reduction at the three determinations on each drug. The statistical significances are shown as ns (= not significant) or by *p*-values.

Acta med. scand. Vol. 193 pp 553–555 1973

HYPOTHYROIDISM FOLLOWING LITHIUM TREATMENT

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Abstract. Three patients with hypothyroidism, who were treated with lithium at the time their disease was discovered, are presented. The possible connection between their hypothyroidism and lithium medication is discussed.

Lithium salts have had an established position among the drugs used in the management and prophylaxis of manic-depressive psychosis since their introduction in 1949 (3) for the treatment of manic patients.

Since the end of the 1960's reports have been published on the development of goitre in conjunction with lithium carbonate medication.

Three cases of hypothyroidism have been referred to the Department of Medicine, Serafimerlasarettet, which were being treated with lithium at the time hypothyroidism was discovered. Their medical histories are related and the possible connection between their hypothyroidism and lithium medication is discussed.

CASE REPORTS

Case 1

Female, 63 years, previously in good somatic health, with the exception of surgery for appendicitis, uterine prolapse and varices.

A manic-depressive psychosis, dominated by depressive episodes, made its debut in conjunction with her menopause at the age of 46 and led to treatment at psychiatric clinics on a number of occasions. Lithium medication was initiated for the first time 7 months prior to admission and repeated determinations of serum concentration disclosed a therapeutic level. This treatment was concluded 2 months prior to admission. Moreover the patient was treated for 2 months with amitriptyline (Tryptizol® MSD), 100–150 mg/day.

The patient was admitted under the diagnosis of hypothyroidism as she had been suffering for 2 months from increasing hoarseness, fatigue, habitual chilliness, obstipation and swelling of her face and hands.

Hoarseness, a puffy face with loss of lateral parts of the eyebrows, dry skin and slow Achilles reflexes characterized the patient's condition upon admission. Her pulse rate was 65 beats/min and BP 140/80 mmHg. Palpation of the thyroid gland showed N.A.D.

Laboratory findings confirmed the diagnosis hypothyroidism: T_4 PBI 0.3 γ% (normal 4–8 γ%), T_3 index 0.91 (normal 0.8–1.4), cholesterol 465 mg/100 ml (normal 140–285 mg/100 ml), BMR −19%. Radioiodine uptake was only 1% in 24 hours and urinary excretion 40% in 24 hours. There was no evidence of panhypopituitarism. Normal results were obtained with respect to X-rays of the sella turcica, electrolytes, gonadotropins, 17-ketosteroids, 17-ketogenic steroids and human growth hormone. Routine laboratory tests were also normal. There was no elevated titre of thyroid antibodies.

After administration of thyroid hormones the patient's laboratory status became euthyroid concomitantly with pronounced subjective improvement.

Case 2

Female, 34 years, with a history of nervous complaints since the age of 16 in the form of uneasiness, anxiety and, in recent years, episodes of depression. Goitre was found in childhood. She has suffered from periodic headaches ever since a brain concussion at the age of 17.

In Sept. 1969 the patient was admitted to a psychiatric clinic because of severe anxiety and meprobamate misuse. She was initially treated with amitriptyline, chlorprothixene and oxazepam. Levomepromazine and lithium sulphate were then administered, the patient being discharged with these two drugs 2 weeks after their institution. There is no information on serum lithium levels. As early as 1 week after the start of lithium medication, the patient noted a weight increase, which grew to 10 kg in 1 month. She became hoarse and her singing voice disappeared. She also felt a slight tenderness in the neck. During this period she was troubled by increasing fatigue, obstipation and habitual chilliness, symptoms which had been sporadically manifest for the past 4 years. The patient stopped using lithium on her own initiative after 1½ months because of the aforementioned symptoms.

A study made on an out-patient basis in Aug. 1970 disclosed: ESR 7 mm, T_4 PBI 4.1–3.8 γ%, T_3 index 1.0 and BMR −15%. Radioiodine uptake displayed normal values but a low T_3 index, possibly suggesting a defect in the synthesis of the thyroid hormones. She then had mildly

carried out, followed by mild hypoglycaemic attacks. The second patient was an insulin-treated diabetic who had four episodes of severe hypoglycaemia after propranolol. Rabkin et al. (10) experienced hypoglycaemic coma of unknown cause in an elderly lady receiving propranolol 80 mg daily but further details are lacking. Mackintosh (9) described a clearcut case of hypoglycaemia provoked by propranolol in a 9-year old girl. She underwent minor surgery preceded by a 20-hour fast. At operation she received propranolol 2 mg i.v. During transfer back to the ward she had grand mal convulsion which responded to an i.m. injection of phenobarbitone. Blood sugar at that time was 12 mg/100 ml. After a 24-hour fast hypoglycaemia was reproduced with [illegible] mg propranolol i.v.

That large doses of propranolol may induce hypoglycaemia is indirectly suggested by a study by Kosinski et al. (6), who reported a 37-year-old female who had ingested a large amount of imipramine, a questionable dose of diazepam and 800 mg propranolol. According to the authors' description the clinical picture was compatible with hypoglycaemia, but unfortunately blood sugar values are not reported. Initial treatment with isoproterenol was unsuccessful, but a marked improvement was seen after i.v. glucagon. Although not discussed by the authors, we think that the main complication in their patient was hypoglycaemia.

Compared with the patient of Mackintosh (9) ours were younger, not fasting, and had received a considerably higher dose of propranolol.

The boy in our report had a long-standing sinus bradycardia indicating severe β-blockade whereas the girl had normal heart rate. This suggests that the boy took the majority of the propranolol tablets. The bradycardia required no treatment. The bradycardia is important from the point of view that it may complicate the diagnosis by obscuring the tachycardia normally seen in hypoglycaemia. The bradycardia and the other ECG abnormalities mentioned above are well known effects of propranolol. The transient rise of BP in the boy remains unexplained.

Propranolol is widely used in clinical practice. The occurrence of propranolol-induced hypoglycaemia is apparently rare, but the possibility should be borne in mind, especially in cases of propranolol intoxication in insulin-treated patients, fasting people and small children.

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medication to develop not only goitre possibly with low PBI values, but also hypothyroidism. The clinical picture in two of the patients described (nos. [illegible] and 3) resembled that of thyroiditis. However none of the patients had typical clinical manifestations of Hashimoto's thyroiditis or De Quervain's thyroiditis. There was indeed an elevated titre of thyroid antibodies in one of the patients (no. 2), but a normal ESR and electrophoresis plus the PAD picture did not point to Hashimoto's thyroiditis. In the second case (no. 3) the PAD picture displayed diffuse lymphomatous, Hashimoto-type thyroiditis, but an essentially normal ESR, normal electrophoresis and the absence of thyroid antibodies again failed to support this diagnosis. Thus, on the basis of two of the cases described, it seems possible that lithium may give rise to a picture resembling thyroiditis. Shopsin et al. (15) were doubtful about the possibility of any connection between lithium intake and the occurrence of thyroid antibodies, but mentioned the possibility that subclinical thyroiditis might be exacerbated and made manifest by lithium medication. This theory would explain why only a few lithium-treated patients develop hypothyroidism. Halberg et al. (8) could not verify an increment of thyroid antibodies but did find an increment of antinuclear cells in patients on lithium treatment.

The described initial, transient low PBI values in connection with lithium medication (4 11 14) were not found in two of the cases related here (nos. 1 and 2). Both patients required thyroid substitute for a long period.

It will probably be necessary to follow thyroid function both before and after lithium medication in order to establish any possible connection between lithium medication and prolonged hypothyroidism.

ADDENDUM

Since the three cases described here were put together patients on lithium treatment with clinical hypothyroidism have been reported in Lancet, one case by Myers (June 10, 1972, p. 1277) and two cases by Rennie and Lazarus (July 1, 1972, p. 44).

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Acta med. scand. Vol. 193 pp. 561–564 1973

DIPHENYLHYDANTOIN HALF LIFE IN MAN AND ITS INHIBITION BY PHENYLBUTAZONE. THE ROLE OF GENETIC FACTORS

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Abstract. The diphenylhydantoin serum half-life (DPH $T/2$) has been determined in 14 pairs of healthy young male twins, 7 identical and 7 fraternal. The mean intrapair variance was significantly less in identical than in fraternal twins, indicating that the individual variability in DPH $T/2$ is mainly due to genetic factors. A daily dose of 100 mg phenylbutazone for 5 or 6 days to 11 pairs of the twins greatly prolonged the DPH $T/2$. A significant correlation between the prolongation of the DPH $T/2$ and the serum phenylbutazone levels was found ($r = +0.586$, $p < 0.01$). Nevertheless large individual variations in the prolongation of DPH $T/2$ at identical phenylbutazone levels ere demonstrated. There was, however no difference in the mean intrapair variance of identical and fraternal twins and we conclude that the individual variability in the extent of impairment of DPH metabolism by phenylbutazone cannot be attributed to genetic influence.

The rate of metabolism of many drugs varies considerably between individuals. In twin studies it has been demonstrated that variations in plasma half-lives of phenylbutazone, dicoumarol, anti pyrine and ethanol were genetically determined (10, 11 13).

Individual variability is also observed in the degree of enhancement of the biotransformation of drugs caused by inducing agents. Vesell and Page (12) showed that the phenobarbital-induced shortening of plasma antipyrine half-life was genetically controlled.

Inhibition of the metabolism of one drug by another is a different type of drug interaction. In this paper we report a large indi idual aria-bility in phenylbutazone inhibition of diphenyl-hydantoin (DPH) metab r E f twin study we have in es g ed e c genetic factors on thi ar on r w ha e found a significan g netu cr diphenylhydantoin half-life DPH T ug free individuals.

SUBJECTS AND METHODS

Fourteen pairs of male twins from the Copenha ea aged 19–25 years, volunteered to this study. The ames were obtained from file of twins ascertained du ing enlistment for military service. All twins appeared to be in excellent health. Determination of at least 20 blood, serum and enzyme characteristics (determined by K. Henningsen, the Institute of Forensic Medicine, University of Copenhagen) divided the twins into 7 pairs of identical twins and 7 pairs of fraternal twins.

W obtained careful drug history None ere abusers of alcohol nor had any of the twins taken drugs for at least 3 weeks before the start of the experiment.

DPH $T/2$ as estimated by injection of 100 mg DPH to which 20 µCi [14]C-diphenylhydantoin (New England Nuclear Corp., Boston, Mass.) had been added. Blood samples were drawn 3, 6, 9 and 12 hours later. The radioactivity was measured as described previously (8). The recovery and reproducibility f the extraction procedure were found to be 88.4% S.E.M. 0.4 = 12. The analytical determinations of the DPH $T/2$ were made in duplicate. The coefficient of variation was 0.07 in the interval from 7–19 hours and 0.12 in the interval from 19–31 hours. The $T/2$ was found by plotting the values in semilogarithmic system. The apparent volume f distribution (V) was calculated from the total number of counts of the injected DPH and the extrapolated serum radioactivity at time zero. On each of the 5 days following the first DPH $T/2$ determination the volunteers took 100 mg phenylbutazone in the morning. Pairs 1 2 and 8 took larger dose (Table I) and pair 3 took 100 mg for 6 days. The day after the last dose had been taken second DPH $T/2$ was done. At this time the phenylbutazone concentration in serum as measured in

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Table I *DPH T/2 before and after phenylbutazone administration in 28 male twins*

Twin no.	Age (y)	DPH T/2 (h) Before phenyl-butazone	DPH T/2 (h) After phenyl-butazone	Increase in T/2 produced by phenylbutazone (%)	Total phenyl-butazone dose	Serum phenylbutazone levels after phenylbutazone (μg/ml)
Identical twins						
1	21	9.8	72.3	638	0.3 g for 10 d.	115
b	21	8.4	36.5	335	0.3 g for 10 d.	128
2a	26	9.7	57.3	491	0.3 g for 9 d.	108
b	26	9.5	42.7	350	0.3 g for 9 d.	107
3a	26	11.5	28.8	150	0.1 g for 6 d.	46
b	26	12.0	25.0	108	0.1 g for 6 d.	48
4a	23	17.0	24.5	44	0.1 g for 5 d.	28
b	23	21.4	22.5	5	0.1 g for 5 d.	36
5a	19	16.1	20.9	30	0.1 g for 5 d.	33
b	19	14.7	31.1	112	0.1 g for 5 d.	39
6	25	12.3	20.4	66	0.1 g for 5 d.	31
b	25	11.7	16.8*	44	0.1 g for 5 d.	27
7	24	25.5	30.1	18	0.1 g for 5 d.	35
b	24	22.2	28.1	27	0.1 g for 5 d.	24
Fraternal twins						
8	23	16.1	75.0	366	0.3 g for 14 d.	140
b	23	9.4	32.2	243	0.3 g for 14 d.	127
9	24	8.1	14.4	78	0.1 g for 5 d.	48
b	24	8.8	14.5	65	0.1 g for 5 d.	33
10	23	11.7	17.8	52	0.1 g for 5 d.	40
b	23	22.0	24.5	11	0.1 g for 5 d.	28
11	24	10.3	22.3	117	0.1 g for 5 d.	38
b	24	13.0	22.4	72	0.1 g for 5 d.	32
12	24	20.7	29.8	44	0.1 g for 5 d.	37
b	24	13.2	20.9	58	0.1 g for 5 d.	41
13a	25	13.8	14.6	6	0.1 g for 5 d.	31
b	25	14.6*	21.2	45	0.1 g for 5 d.	33
14	24	11.6	19.6	69	0.1 g for 5 d.	31
b	24	7.7	12.8	66	0.1 g for 5 d.	28

* Single determination.

duplicate by the method of Burns et al (1). Ultrafiltration studies were performed as earlier described (8). Phenylbutazone, 50 μg/ml, did not change the percentage of non-protein-bound DPH (9.9% vs 9.6%). The concentration of DPH employed was 33 μg/ml.

RESULTS

Diphenylhydantoin half-life

The DPH $T/2$ values before phenylbutazone are listed in Table I. From these the mean intrapair variance of the two groups of twins was calculated as the total intrapair variance divided by twice the number of pairs of twins. In the group of fraternal twins a value of 16.49 was found and in the identical twins the variance was 2.49. An F test showed these variances to differ significantly ($p < 0.05$). A heritability index was calculated according to Osborne and DeGeorge (9): variance within pairs of fraternal twins minus variance within pairs of identical twins/variance within pairs of fraternal twins. A value of 0.85 was obtained, indicating a strong genetic influence on DPH $T/2$ in man. Fig. 1 shows typical examples of DPH $T/2$ in two pairs of identical and two pairs of fraternal twins.

Lengthening of diphenylhydantoin half-life by phenylbutazone

Table I also shows the levels of serum phenylbutazone and the DPH $T/2$ values after administration of phenylbutazone. The prolongation of half lives due to phenylbutazone was submitted to statistical analysis as outlined above. There was no difference in the mean intrapair variances of identical and fraternal twins receiving a total dose of 500 mg (600 mg for pair 3),

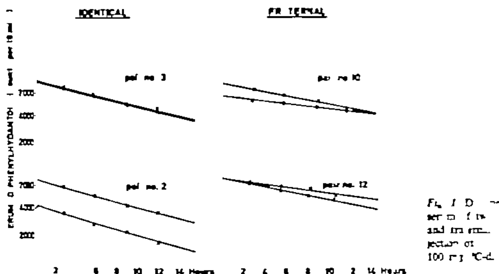


Fig. 1. D... ser... and fra... jection of 100 mg ¹⁴C-d...

regardless of the prolongation (calculated per µg phenylbutazone per ml of serum) expressed in hours or percental change from the initial half life determinations. Fig. 2 shows the half-lives of one pair of identical and one pair of fraternal twins before and after phenylbutazone administration.

The mean initial DPH $T/_2$ in all twins was 13.7 h, S.D. 4.8 h (range 7.7–25.5 h). After phenylbutazone, 500 mg orally, the mean serum concentration of phenylbutazone was 33.8 µg/ml, S.D. 5.7 (range 24–48 µg/ml) and the mean DPH $T/2$ 22.0 h, S.D. 5.5 h (range 1..8–31.1 h).

The apparent volume of distribution of DPH in all twins before phen lbutazor S.D. 10.3 l. Afte phenylbutazone it 57.5 l, S.D. 10.3 l ($p < 0.001$). There nificant difference in the mean intrapair ... of identical and fraternal twins concerning the change in $T_{½}$ of DPH after phenylbutazone.

DISCUSSION

Our results show that persons not ingesting other drugs eliminate DPH at a rate mainly controlled by genetic factors. The relative contribution of heredity is of the same order of magnitude as that described for halothane metabolism (2), but con-

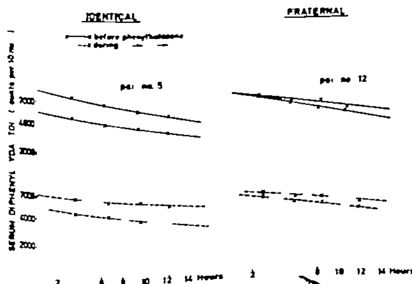


Fig. 2. Change in serum DPH $T/2$ before and after phenylbutazone, 100 mg daily for 5 days, in one set of identical and one set of fraternal twins.

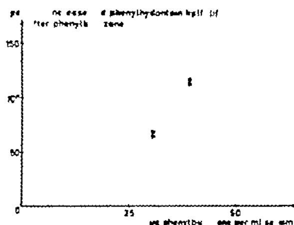


Fig 3 Relationship between percental increase in serum DPH T/2 and serum phenylbutazone levels. Note the large individual variation in DPH T/2 increase at identical serum phenylbutazone levels.

siderably smaller than found for dicoumarol, antipyrine phenylbutazone and ethanol (10, 11 13)

Prolongation of DPH T/2 by phenylbutazone was first reported by Hansen et al (6). We find a correlation between the prolongation of the DPH T/2 and the serum phenylbutazone concentrations, calculated on all data except from pairs 1 2 and 8 ($r = -0.586$, $p < 0.01$) (Fig 3). Nevertheless large individual variations in the prolongation of the DPH T/2 are observed at identical phenylbutazone levels. Individual variability in the extent of allopurinol and nortryptiline inhibition of antipyrine and dicoumarol half lives has previously been reported (14) However blood levels of allopurinol and nortryptiline were not measured.

Experimental evidence suggests that phenylbutazone inhibits microsomal enzymes in the liver (3 5) but this is only a transient effect soon followed by induction. Phenylbutazone and diphenylhydantoin are hydroxylated in the drug-metabolizing microsomal system in the liver. Probably diphenylhydantoin phenylbutazone and its metabolite, oxyphenylbutazone, compete for the limited capacity of this system. Unpublished experiments in our laboratory have shown that oxyphenylbutazone also prolongs the DPH T/2 in man (7)

We have failed to demonstrate that genetic factors control the individual variability in the extent of inhibition observed when the DPH T/2 is measured at identical phenylbutazone levels in the blood. Our findings may suggest that lipid-soluble compounds in the environment (4) interfere with microsomal oxidation of drugs in the liver

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Acta med. scand. Vol. 193 pp. 565–571 1973

PRIMARY AMENORRHOEA WITH HYPERTENSION DUE TO 17 HYDROXYLASE DEFICIENCY

Therapy with Dexamethasone and Ethinyloestradiol

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Abstract. Endocrine studies in girl with 17-hydroxylase deficiency are described. Administration of 0.75 mg dexamethasone was sufficient to make the hypertension and electrolyte disturbances disappear. The capacity to secrete aldosterone is probably unimpaired in this patient, but seemed completely suppressed before treatment with dexamethasone and subnormal during administration of 0.75 mg daily of this steroid, though plasma renin activity (PRA) became normal. Addition of cyclical ethinyloestradiol to the dexamethasone treatment resulted in elevated PRA levels and again rise in blood pressure.

Since the publication by Biglieri et al. in 1966 (3) concerning a girl with congenital 17-α-hydroxylase deficiency eight other cases have been described (12, 15, 16, 17, 19, 22, 24). Two of them were sisters (16), two patients were male (17, 22). A similar abnormality was found in an infant with a corticosterone producing tumour (29). In 17-α-hydroxylase deficiency the production of 17-α-hydroxyprogesterone and 17-α-hydroxypregnenolone is decreased. Secretion of 11-desoxycortisol and cortisol is diminished and production of androgens and oestrogens of adrenal and gonadal origin may also be impaired. In boys this enzyme defect induces male pseudo-hermaphroditism, girls have a normal phenotype with primary amenorrhoea and absence of secondary sexual characteristics. In all cases except one (22) hypertension and slight to moderate hypokalaemic alkalosis was observed. We studied a girl suffering from this syndrome before and after 3 months treatment with dexamethasone and again after 3 months treatment with dexamethasone and cyclical administration of ethinyloestradiol. Dexamethasone without dietary salt restriction reduced blood pressure to normal within 2 months, but after addition of ethinyloestradiol it again showed some tendency to rise.

CASE REPORT

A 20-year-old girl presented with primary amenorrhoea. She had not suffered from any serious illness. Her mother had her menarche at the age of 14. She has no sisters; one brother aged 23 is normal. Physical examination showed well proportioned girl of infantile habitus. Height 1.67 m, span 1.69 m, weight 61 kg. BP varied between 160/100 and 170/125 mmHg. Secondary sexual characteristics were totally absent. The vagina was normal, uterus and adnexa were not palpable.

On ophthalmological examination hypertension grade II (Keith-Wagener) was found. ECG showed left axis deviation with left ventricular hypertrophy. Roentgenologically the heart was of normal size. The sella turcica was normal. The bone age was 13 years (Greulich and Pyle). Intravenous pyelogram and renogram were normal.

Hb 14.1 g/100 ml, haematocrit 40 100 ml, WBC 6 800/mm³. Urinalysis normal, culture negative. Alkaline phosphatase 3.2 U (Bessey), SGOT 10 U, SGPT 12 U, calcium 9.4 mg/100 ml, phosphorus 3.9 mg/100 ml, total protein 6.9 g/100 ml with normal electrophoretic pattern. Serum sodium 142–148 mEq/l, potassium 2.5–3.0 mEq/l, chloride 103–105 mEq/l, blood urea 15–21 mg/100 ml, serum creatinine 0.6–0.8 mg/100 ml, 24 h endogenous creatinine clearance 88 ml/min. Arterial blood pH 7.48, pCO 39 mmHg, pO_2 79 mmHg, standard bicarbonate 28 mEq/l. PBI 7.6 μg 100 ml, resin T3 uptake 18.2% (normal below 24.0%). Glucose tolerance test 97 mg/100 ml, after / h 142 mg/100 ml, after 1 h 120 mg 100 ml, after 1 / h 11 mg 100 ml, after h 135 mg 100 ml, after 2 / h 102 mg 100 ml.

Culture of the white blood cells revealed normal female karyotype 46 XX, dermatoglyphics were normal.

The patient as treated first with dexamethasone 0.25 mg in the morning and 0.50 mg in the evening for 3 months, and thereafter with combination of dexa-

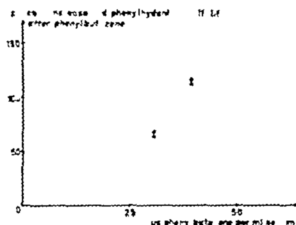


Fig 3 Relationship between percental increase in serum DPH *T*/2 and serum phenylbutazone levels. Note the large *individual ariation in DPH T increase at identical* serum phenylbutazone levels.

siderably smaller than found for dicoumarol, antipyrine phenylbutazone and ethanol (10 11 13).

Prolongation of DPH *T*/2 by phenylbutazone was first reported by Hansen et al (6) We find a correlation between the prolongation of the DPH *T*/ and the serum phenylbutazone concentrations, calculated on all data except from pairs 1 2 and 8 ($r = +0.586$, $p < 0.01$) (Fig. 3). Nevertheless large individual ariations in the prolongation of the DPH *T* are observed at identical phenylbutazone levels Individual variability in the e tent of allopurinol and nortryptiline inh bition f ant pyrine and dicoumarol half l es has previously been reported (14) However blood le els of allopurinol and nortryptiline were not measured

Experimental eviden e suggests that phenylbutazone inhibits microsomal enzymes in the li er (3 5) but this is only a transient effect soon followed by induction Phenylbutazone and diphenylhydantoin are hydroxylated in the drug metabolizing microsomal system in the liver Probably diphenylhydantoin phen lbutazone and its metabolite oxyphenylbutazone, compete for the limited capacity of this system. Unpublished experiments in our laboratory have shown that *oxyphenylbutazone also prolongs the DPH T/2* in man (7)

We have failed to demonstrate that genetic factors control the individual variability in the extent of inhibition observed when the DPH *T*/2 is measured at identical phenylbutazone levels in the blood. Our findings may suggest that lipid soluble compounds in the environment (4) interfere with microsomal oxidation of drugs in the liver

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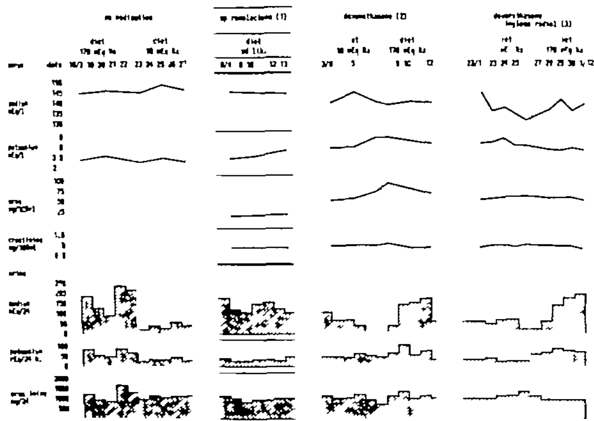


Fig. 1. Summary of electrolyte studies. (*1*) spironolactone 200 mg q.i.d., (*2*) dexamethasone 0.25 mg at 8 h and 0.50 mg at 17 h (*3*) dexamethasone 0.25 mg at 8 h and 0.50 mg at 17 h, ethinyloestradiol 30 μg daily for 3 weeks with 1 week's intermission.

according to Appleby et al. (1). Urinary pregnanediol and pregnanetriol were measured according to Bongiovanni and Clayton (6). Adrenocortical hormones and their metabolites were determined according to Cost and Vegter (11), aldosterone according to Neher and Wettstein (21). Oestrone, oestradiol and oestriol were measured by the method of Brown (7).

Cortisol in blood was determined according to Sweat (28), corticosterone as described by Artz (2). Cortisol secretion rate was measured according to Cope and Black (10). Progesterone was measured with competitive protein binding technique (14), serum luteinizing hormone was measured by radioimmunoassay using the 2nd International Reference Preparation of Human Menopausal Gonadotrophin as standard. The last two determinations were performed commercially by Searle Scientific Services, High Wycombe, Bucks, England.

Plasma renin activity (PRA) was determined as described by van der Meer (18) by the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, by radioimmunoassay of angiotensin I.

The patient was on restricted sodium diet, 170 mEq sodium or ad lib. diet as shown in Tables I and II and Fig. 1, in which the medications and dosages of ACTH, dexamethasone and ethinyloestradiol are also indicated.

All hormone studies were done at the end of an experimental period.

RESULTS

The urinary steroid excretion is shown in Table I, the hormone studies in blood in Table II and the electrolyte data in Fig. 1. A schematic representation of some steroid interconversions is shown in Fig. 2.

Glucocorticoids and progesterone

Plasma cortisol in our patient was extremely low: cortisol secretion rate was lower than 0.5 mg/24 h. Administration of 0.15 mg tetracosactide in a 3 h i.v. infusion resulted in a plasma cortisol of 2.4 μg/100 ml. Stimulation with 0.25 mg tetracosactide in 5% glucose solution during 8 h on two subsequent days was without effect on urinary cortisol and cortisol metabolites. The excretion of pregnanetriol was low normal, as is usual in

the patient had been kept on the same diet for a longer period. The ability of her adrenals to synthesize aldosterone is amply proven by the marked increase of aldosterone excretion in the low salt period during combined therapy with dexamethasone and cyclical ethinyloestradiol. It should, however be realized that the above reasoning is only valid if the excretion of aldosterone and its 3-oxyconjugate follows actual aldosterone production in the various experimental conditions.

BP fell during treatment with dexamethasone in 2 months and the metabolic alkalosis disappeared. Addition of ethinyloestradiol induced a rise of PRA, a rise of aldosterone excretion and of the metabolites of corticosterone. Mild hypertension recurred. This situation could parallel the hypertension that is found in women using oral contraceptives, in whom PRA is not suppressed, as the substrate increases because of oestrogen administration (25). It is possible that increased PRA would not only stimulate aldosterone production but also the secretion of corticosterone since angiotensin in vitro exerts its action early in steroid biogenesis, probably at the site of conversion of cholesterol to Δ5-pregnenolone (9). It has been suggested that this also occurs in vivo with physiological levels of angiotensin (4). Following this train of thought it could be surmised that the hypercorticosteronism of our patient in the pretreatment period was caused by high levels of plasma ACTH. This, in effect, was found in the ses in which it was studied (1 3). The presence of slightly elevated excretions of corticosterone metabolites during treatment with dexamethasone/ethinyloestradiol might then be caused by a disordered renin/angiotensin mechanism, as is sometimes seen in women on oral contraception.

A clinical picture without hypogonadism has been described in four patients, three of them men (20, 23 27), in whom a low cortisol secretion rate was found with depressed PRA and high secretion of desoxycorticosterone but also elevated aldosterone production and normal excretion of pregnanetriol. In two of these patients elevated plasma levels of ACTH were found (23 27). Administration of dexamethasone normalized all biochemical abnormalities. An explanation of this syndrome could be relative 17-hydroxylase deficiency (20 23) causing a less pronounced shift of cortisol to corticosterone production in the adrenals.

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Acta med. scand. Vol. 193 pp. 573–576 1973

WALDENSTRÖM'S MACROGLOBULINAEMIA WITH XANTHOMATOSIS AND HYPERCHOLESTEROLAEMIA

Report of a Case

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Abstract Even though most cases of M-globulinaemia reveal low serum cholesterol values, few cases are on record in which hypercholesterolaemia and/or xanthomatosis have been associated with IgG or IgA paraproteinaemia. In most of the latter cases familial origin of the disease was not disclosed, and causal relationship between the paraproteinaemia and the lipoid disease was therefore assumed. A report is presented of case associated with Waldenström macroglobulinaemia—a combination which does not seem to have been described before. This case probably represents fortuitous coincidence of the two diseases, as electrophoresis of the serum lipoproteins in the patient' sister revealed lipoid changes, although this sister had no xanthomata and her serum cholesterol level was only at the upper limit of normal. Thus familial genesis can be excluded only by an analysis of serum lipoids in members of the family

Low serum cholesterol levels are often found in patients with myelomatosis and macroglobulinaemia (7 13 17 18). This is in harmony with the fact that autopsy rarely reveals atherosclerosis in such patients (13 14).

However Snapper and Kahn (13) mentioned 11 cases of hypercholesterolaemia and/or xanthomatosis in myelomatosis or non-myelomatous monoclonal M-globulinaemia which had been published within a 15-year period. In all 11 cases a sharply demarcated M-globulin was demonstrated (IgA, 7 IgG 2, type of M protein not stated, 2 cases).

In 1970 Ozer et al. (19) reported an additional case of myelomatosis associated with hyperlipidaemia, at the same time referring to 19 cases of this combination previously published in the literature, but still no cases in which the paraprotein was IgM were on record. Twelve of the patients had typical xanthomatous skin lesions, while four had xanthomata without hyperlipidaemia. In two of the patients familial xanthomatosis was demonstrated, which suggested a fortuitous occurrence of the lipoid disease and myelomatosis (4 8).

Even though the rule seems to be that the serum cholesterol level is reduced in paraproteinaemia, the problem arises whether there may occasionally be a causal relation between M-proteinaemia, hypercholesterolaemia and xanthomatosis. In none of the above mentioned cases of this combination was the M component an IgM. This has prompted us to report such a case in an attempt to contribute to the sol tion of the problem whether a causal relation exists or whether the combination is due to mere coincidence.

CASE REPORT

Our patient was 64-year-old man, admitted to hospital with lobar pneumonia. During his stay in hospital (Dec. 1965) macroglobulinaemi was diagnosed. ESR 115 mm/h, total serum protein 8.4 g/100 ml with an M component of 1.8 g. The Sia test was positive. Immunoelectrophoresis of the serum showed that the M protein was an IgM with β-2 mobility. The bone marrow was hypercellular with 25–30% lymphocytes, of which few were plasmocytoid, and 3–4% morphologically normal plasma cells. Bone destruction, proteinuria and Bence Jones protein were absent. Hb level 12 g 100 ml, leukocytes 6 000/µl with normal distribution, platelets 200 000/µl; the erythrocytes from peripheral blood showed rouleaux formation.

Renal function was normal, serum thymol turbidity increased, other liver tests normal; no glucosuria.

In the serum of three patients with both myelomatosis and hyperlipaemia Spikes et al. (15) demonstrated the presence of a substance which in in vitro experiments was "capable of so altering serum beta lipoproteins of normal sera that these lipoproteins would not migrate in a starch-gel system" The serum factor responsible for this lipoprotein alteration was isolated, characterized and identified. It was the myeloma paraprotein. However they added that "the nature of this in vitro alteration is unclear and it remains to be established what role, if any this in vitro activity has in promoting the hyperlipemia seen in these persons"

Stauffer et al. (16) discussed the presumed relation between hyperlipaemia and malignant disorders other than myelomatosis on the basis of their observations in two children suffering from monocytic leukaemia and lymphosarcoma, respectively The leukaemic patient had also monoclonal gammapathy In these two patients the exclusion of the familial nature of the hyperlipaemia was based only on the medical history The authors suggested that, in some cases of the combination of malignant disease and hyperlipaemia, it would be reasonable to consider whether the latter condition may be a 'mobilization or transport hyperlipaemia' which may be the explanation if it does not become manifest until in the terminal cachectic phase.

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